Neuromechanic Aspects of Desert Ant Navigation

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1 Zusammenfassung / Summary

Zusammenfassung

Die aussergewöhnlichen Naviationsleistungen der Wüstenamseise *Cataglyphis fortis* beruhen auf egozentrischer Navigation, also der Integration des gelaufenen Weges und der gleichzeitigen Berechnung der direkten Verbindung von der aktuellen Position zum Startpunkt (Nest). Zur Berechnung des Heimvektors muss *Cataglyphis* von jedem einzelnen Pfadsegement die Richtung, die Länge sowie die Steigung dieses Segments bestimmen. Die Richtungsbestimmung erfolgt mit Hilfe des sogenannten Himmelskompasses, der den Stand der Sonne sowie das Polarisations- und Spektralmuster auswertet. Im Gegensatz dazu werden Enfernungen über einen Schrittintegrator bestimmt, der die Eigenbewegungen der Beine während des Laufens auswertet. Auch Steigungen erfasst *Cataglyphis* nach derzeitigem Stand der Forschung idiothetisch; doch ist auch hier der genaue Mechanismus noch unbekannt. Damit bilden die biomechanischen Aspekte der Navigation von *Cataglyphis fortis* die zentrale Fragestellung dieser Arbeit.

Visuelles und taktiles Lernen von Bodenstrukturen Der erste Hauptteil der Arbeit (Kapitel 3) behandelt die mechanosensorischen Leistungen der Tarsi. Es gelang der Nachweis, dass *Cataglyphis* lokale taktile Eigenschaften des Untergrunds lernen und in einer Testsituation abrufen kann. Wenn zum Beispiel die unmittelbare Umgebung um den Nesteingang eine besondere, vom übrigen Gelände abweichende Rauhigkeit besitzt, wird die Nestsuche im Testkanal auf eine analoge rauhe Stelle konzentriert, auch wenn dieser Ort mit dem Vektor-bestimmten Nestort nicht übereinstimmt. Taktil wahrnehmbare Boden-Landmarken behandelt *Cataglyphis* gleichwertig wie die bisher bekannten rein visuell perzipierten Panorama-Landmarken. Doch müssen bei den Bodenlandmarken

in der Testsituation sowohl die taktilen als auch die visuellen Reize mit den gelernten übereinstimmen, um im Test von den Ameisen akzeptiert zu werden.

Aktives Laufen als Voraussetzung der Wegintegration Während fliegende Insekten Entfernungen mit Hilfe des wahrgenommenen optischen Flusses messen, spielt visueller Input bei den laufenden Wüstenameisen nur eine untergeordnete Rolle. Stattdessen sind idiothetische Stimuli an der Entfernungsmessung beteiligt. In Kapitel 4 wird untersucht, ob die Bedeutung von visueller Information zunimmt, wenn der Eingang idiothetischer Information unterbrochen ist. Die Ergebnisse zeigen klar, dass auch in diesem Fall visuellen Stimuli keine Bedeutung zukommt. Aktives Laufen ist eine unabdingbare Voraussetzung für erfolgreiche Wegintegration und die daran beteiligte Entfernungsmessung.

Mechanismen der idiothetischen Steigungs- und Entfernungsmessung Kapitel 5 beschäftigt sich mit den Mechanismen, die der idiothetischen Entfernungsmessung und der Steigungsmessung zugrunde liegen. Die bisher gewonnenen Erkenntnisse lassen drei verschiedene Systeme der Schrittlängen- und Steigungsmessung diskutabel erscheinen: (i) Der Arbeitsbereich der Coxalgelenke könnte sich in Abhängigkeit von den Laufbedingungen (Steigung: parallele Verschiebung der Aufsetz- und Abhebepunkte talwärts; Geschwindigkeit: Erweiterung bzw. Verkleinerung des Arbeitsbereichs der Coxal-Gelenke) verändern. (ii) Die geometrischen Verhältnisse der Schrittlänge und Extrempunkte beim Laufen auf verschieden geneigten Ebenen könnten konstant bleiben, doch die zeitlichen Verhältnisse der einzelnen Phasen des Schrittzyklus sich derart verschieben, dass sich aus diesen zeitlichen Veränderungen die gelaufene Steigung ablesen liesse. (iii) Alle diese Bewegungsparamter könnten steigungsinvariant sein, aber die während der Lokomotion wirkenden Kräfte je nach Laufbedinung variieren und somit als Korrelat für die gelaufene Steigung bzw. Schrittlänge dienen.

Als klares Ergebniss umfangreicher hochfrequenz-kinematographischer Analysen und parametrischer Auswertung zeigt sich, dass die geometrischen und zeitlichen Abläufe der Ameisenlokomotion sind bei verschiedenen Steigungen konstant bleiben. Lediglich die vom Tier gewählte Geschwindigkeit variiert. Da jedoch auf geneigten Flächen die jeweils zur Laufrichtung parallele Komponente der Schwerkraft auf den Ameisenkörper bremsend

oder beschleunigend wirkt, müssen für gleiche Bewegungsabläufe jeweils unterschiedliche Bodenreaktionskräfte erzeugt werden. Begrenzt man - wie im zweiten Experiment dieses Kapitels gezeigt - diese Bodenreaktionskräfte künstlich, kann *Cataglyphis* von aussen einwirkende Kräfte (z.B. Wind, Schwerkraft) nur noch in bestimmten Situationen kompensieren. Daher wird die Hypothese formuliert, dass das (hier nur qualitativ bestimmte) Produkt aus horizontal übertragener Kraft und Anzahl produzierter Schritte während des Auslaufs Einfluss auf die Lage des Suchzentrums der Tiere während des Rücklaufs nimmt.

Schlussfolgerungen

1. *Cataglyphis fortis* ist in der Lage Bodenrauhigkeiten taktil mit den Tarsen zu erfassen und als Navigationshilfe zu benutzen.
2. Im Gegensatz zu rein visuell perzipierten Panorama-Landmarken sind bei Bodenlandmarken sowohl die visuellen als auch die taktilen Eigenschaften perzeptorisch von Bedeutung.
3. Idiothetische Reize bilden die Haupteingangsgrössen für den Mechanismus der Entfernungsmessung bei *Cataglyphis*. Werden sie experimentell ausgeschaltet, haben auch visuelle Reize keinen Einfluss mehr auf den Wegintegrator.
4. Das Laufverhalten der Ameisen ist in allen untersuchen Parametern (Schrittlänge, Geometrie der Extrempunkte, Schrittfrequenz, Zeit von Protraktionsphase und Retraktionsphase, Beinkoordination) steigungsinvariant. Steigungsmessung kann somit nicht anhand von positionssensitiven Sensoren in den Beingelenken vorgenommen werden.
5. Auf glatten Oberflächen wird das sonst stets feste Verhältniss von Schrittlänge und Schrittfrequenz entkoppelt. Trotzdem kann *Cataglyphis* bei Windstille und starkem Gegenwind gelaufene Distanzen korrekt messen.
6. Aus den letzten beiden Ergebnissen folgt, dass Kraftsensoren in der Kutikula der Beine (wahrscheinlich die campaniformen Sensillen) eine wichtige Rolle bei der Messung von Steigungen und Schrittlängen spielen müssen.

Summary

Desert ants of the genus *Cataglyphis* accomplish extraordinary navigational tasks based on egocentric navigation, i.e. the integration of the path covered and the simultaneous computation of the direct route back to the start (nest). In order to determine the home vector *Cataglyphis* has to acquire direction, length, and inclination of each path segment covered. While directional information is derived from skylight cues (position of the sun, polarization and spectral patters in the sky), distances are measured via a step integrator, which depends on analyzing idiothetic cues derived from the movements of the legs. Similarly, inclinations are monitored by idiothetic cues, but again, the exact mechanism is so far unknown. Hence, this thesis focusses on the biomechanic aspects of *Cataglyphis* navigation.

Visual and tactile learning of ground structures The first study of the present account (chapter 3) deals with the mechanosensory performances of the tarsi. It is shown that *Cataglyphis fortis* can learn local tactile properties of the substrate and recall these memories during test situations. If, for example, the close surroundings of the nest entrance are characterized by a particular surface roughness, in the test situation the ant will focus its nest search behavior on a spot within the experimental channel array that contains just this particular kind of surface roughness, even if this spot does not coincide with the position of the vector-defined goal, the nest entrance. Tactily perceived ground landmarks are treated by the ants equally well as the well known visually perceived panoramic landmarks are, but in this case both tactile and visual cues of the test landmark have to be identical to those of the training landmark.

Active locomotion as a prerequisite for path integration While flying insects such as honey bees monitor distances entirely by exploiting visual flow field cues, walking desert ants use this sensory channel only to a minor extent. Instead, idiothetic stimuli have been shown to be involved in distance estimation. In chapter 4 we probe the navigational relevance of visual stimuli for desert ants under conditions in which the input of idiothetic cues is inhibited. The results clearly show that in such situations visual stimuli are completely disregarded. Active locomotion is absolutely necessary for successful path integration and distance estimation (odometry).

Mechanisms of idiothetic monitoring of inclination and step length Chapter 5 deals with the mechanisms that underlie the idiothetic monitoring of distances and inclinations. On the basis of extensive high-speed video-cinematography and single-frame parametric analyses three theoretical possibilities are discussed: (i) The working area of the coxa-joints could depend on different locomotory conditions (inclination: parallel shift of the touch down and lift off points of the legs; speed: widening or narrowing of the coxal working area). (ii) The geometric relationships of step length and footfall geometry could remain constant, but the temporal patterns of the single phases of the stepping cycle could vary in a way that allowed for the determination of inclination. (iii) All the kinematic parameters mentioned could remain invariant to running on inclines, but in consequence the forces acting during locomotion could vary considerably and thus serve as a correlate for the inclination encountered and the step length performed by the ant.

Indeed, results of the above mentioned analyses show that the geometric and temporal kinematic parameters remain invariant against the angle of inclinations; it is only the speed chosen by the ants that varies. As on inclined surfaces a surface-parallel component of the graviational force is acting on the ant's body, these (invariant) kinematic parameters require a variability in force production. If the ground reaction forces - as done in the second experiment of this account - are artificially limited, the ants can only partially compensate externally acting forces such as wind load (or presumably gravity). The (qualitatively determined) product of horizontally transmitted force and number of steps performed during the outbound run has an influence on the ant's estimation of its starting position during the inbound run.

Conclusions

1. *Cataglyphis fortis* can learn local tactile properties of the substrate and use such memorized cues for navigational purposes.
2. Contrary to purely visually perceived panoramic landmarks, ground landmarks are perceived only by both visual and tactile cues in combination.
3. Idiothetic stimuli are the main cues involved in distance estimation (odometry) in *Cataglyphis* ants. If these stimuli are eliminated visual input will not be used as, e.g., compensatory information.

4. Various parameters of the ant's locomotory behavior (e.g. step length, footfall geometry, step frequency, stance period, swing period, leg coordination) are invariant against varying inclinations of the floor on which the ants are running. In consequence, the ants cannot monitor inclines by using information from their positional-sensitive hair sensilla in the leg joints.
5. On slippery surfaces the otherwise rigidly fixed relationship between step length and step frequency is uncoupled. Nevertheless *Cataglyphis* can estimate distances correctly, even under experimentally induced adverse conditions, i.e. under externally acting forces such as wind or gravity.
6. Based on the last two results it is claimed that force measurements of, for instance, campaniform sensilla in the cuticle of the legs most likely play a key role in the ant's mechanisms of monitoring inclines and distances.

2 Introduction

2.1 General background and thesis objectives

"We think that the nervous system and the mechanical system should be designed to work together, sharing responsibility for the behaviour that emerges" (Raibert and Hodgins, 1993, in Kubow and Full, 1999)

Animal behavior involves both neuronal and mechanical control. The control is achieved by well tuned neuronal circuits that monitor the result of the actions performed by an effector, and elicit appropriate responses. However, the mere mechanical design of any effector, be it leg or wing, plays an important role in successful control. In fast running insects the control of locomotion is partly facilitated by elaborate leg design, incorporating spring-like and damping behavior. Hence, Kubow and Full (1999) called for an approach - called neuromechanics - which dedicates its attention to the interplay of mechanical and neuronal systems, and the behavior emerging from this interplay. Especially fast movements seem to be controlled by a feed-forward manner rather than by sensorial feedback (Full and Koditschek, 1999). In the case of desert ants, *Cataglyphis fortis*, fast movements are essential in the foraging lives of these animals, which on the other hand also need to precisely monitor these fast movements in order to navigate correctly back to the nest. The core motivation of this thesis is elucidating the interplay of mechanosenory input during fast locomotion and the emergent behavior of egocentric navigation.

Active and fast locomotion is one of the most obvious success stories in animal evolution. On land, legged locomotion employed mainly by arthropods and tetrapods provides the fastest and most flexible means of active movement. However, the faster the movement, the more elaborate has the sensing and evaluating of environmental cues to be. Each

running animal has to maintain a course of locomotion and has to recognize and avoid obstacles and predators. Meanwhile it needs to maintain its body in the very distinct position that allows locomotion, i.e. normal to the substrate. Additionally, certain animals maintain a social lifestyle where division of labor is established and only a fraction of the colony provides the food for all the members. In this case survival of the colony is also dependent on highly advanced navigational performances by the individual foragers.

In order to fulfill all these tasks each animal has to monitor and process a multitude of sensorial information. Only if these processes are performed in real-time, the animal can decide on, and initiate appropriate reactions towards, unforeseen situations. However, this computing needs to meet certain potentially contradictory demands: On the one hand there has to be a reduction of the physiological machinery to the absolute necessary, in order to keep energetic investment as low as possible, but on the other hand as much information as possible has to be evaluated in a fast and failure proof way. Such minimal algorithms stand out in terms of short procession time and low resource demands, and yet they are able to cope with unforeseeable situations. Hence, research on animal navigation may provide insight for technical applications, especially in systems that have to function autonomously.

Figure 2.1: The desert ant *Cataglyphis fortis* hurrying off to start a foraging run.

In this respect, one of the most remarkable groups of animals are ants of the genus *Cataglyphis* (Fig. 2.1). These eusocial animals are found in habitats with atrocious living conditions: They inhabit deserts and salt pans (Fig. 2.2), withstand dry and hot climatic conditions, and are specialized to feed only on other arthropods that have

succumbed to the heat and desiccation stress of their desert environment. However, they follow their daily business even at the hottest time of the day performing foraging runs up to 150 meters (or about 150 000 times their body-length; Wehner, 1983) from the nest. Moreover they navigate safely back to their point of departure, where after an extended foraging journey they hit the inconspicuous nest entrance even in the absence of external landmark information. As in desert habitats odor trails would suffer from quick evaporation, the *Cataglyphis* ants employ a highly elaborated means of egocentric navigation (Müller and Wehner, 1988; Wehner and Srinivasan, 2003). While searching for food, they integrate each single step made into the path integrating system and continuously calculate their position in relation to the nest. This type of vector navigation (Wehner, 1982) allows them to return home on a direct path once a food item has been found. In integrating their paths they must monitor the direction, the distance, and - in habitats more structured than salt pans (Fig. 2.2) - the inclination of each incremental segment of their path (Wohlgemuth et al., 2001).

Figure 2.2: Typical habitat of *Cataglyphis fortis* and some ant researchers at our field site in Maharès in the south of Tunisia. (Photo: M. Wittlinger)

While the ants obtain directional information by exploiting skylight cues such as the polarization and spectral patterns in the sky or the position of the sun (e.g. Wehner and Lafranconi, 1981; Wehner, 1989; Wehner 1994a; Wehner and Srinivasan, 2003; Wehner and Labhart, 2006), information on distances covered and inclinations run are subject to idiothetic cues, i.e. monitoring of self movement (see below).

The general focus of the current PhD-thesis are the biomechanical processes involved in

desert ant navigation. Among other aspects, this includes mainly the prerequisites for successful path integration and the biomechanic basis for idiothetic monitoring of step length and surface inclination.

2.1.1 Biomechanics of path integration

As early as 1904 Pieron suggested that ants measure distances by "counting" steps. Although this hypothesis remained untested for long, other hypothesis of distance estimation in ants were brought up - and systematically ruled out (energy expenditure: Schäfer and Wehner, 1993; Wohlgemuth et al., 2001; skylight cues: Wohlgemuth et al., 2002; optic flow: Ronacher and Wehner, 1995; Ronacher et al., 2000). More than 100 years after Pieron's seminal account Wittlinger and co-workers finally showed, while performing an elegant experiment, that ants indeed do integrate steps by some sort of pedometer (Wittlinger et al., 2006; Wittlinger et al., 2007a). However, the ants do not merely count steps but integrate step length as well in order to correctly calculate distances under different locomotor speeds. The mechanism by which step length is monitored is one of the questions I address in this thesis.

The work of Wohlgemuth et al. (2001, 2002) raised an even more challenging question. The authors showed that desert ants can integrate distances they have covered even in the third dimension, obviously monitoring the inclination of the surface on which they run on. Neither skylight cues (Wohlgemuth et al., 2002) nor information about energy expenditure during running (Lipp et al., 2005) are used by the ants in measuring the inclination of the surface on which they walk. In a remarkable early work Markl (1962) showed that ants perceive gravity by hair field sensors arranged between body segments and at leg joints. However, desert ants correctly estimated their homing distance even after hair fields had been deactivated (Wittlinger et al., 2007b). If angles of inclination were monitored by idiothetic means, even after all between-body-segments sensory hair fields had been excluded, the tiny leg based hair fields remain as the only source of information. Locomotion on inclined surfaces has an influence on locomotory behavior simply by the fact that an additional external force, the force component of gravity that runs parallel to the substrate, is acting on the ant's body (Fig. 2.3). In a series of experiments I examined the reaction of the ant's locomotory system on a tilted vector of gravity.

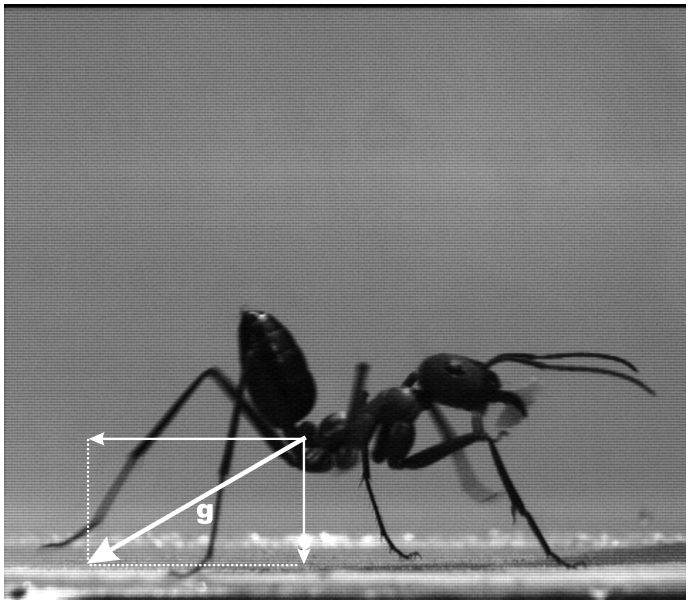

Figure 2.3: Gravity (g) acting on an ant's body when running on an inclined surface (in this case 60°). The tilted gravity vector applies an external force on the ant's body accelerating it backwards.

2.1.2 Biomechanics of landmark navigation

Although *Cataglyphis*-ants can entirely rely on their vector navigation system, they use - whenever possible - landmark information as well. In accordance with the fact that any egocentric path-integration system is prone to cumulative errors, but that a landmark system is not, *Cataglyphis* prefers landmark information over vector-information if both types of information are available but experimentally made to disagree (e.g. Wehner et al., 1996; Bregy and Wehner, 2003; Andel and Wehner, 2004). However, if the landmarks get out of sight, the ants immediately fall back to the information provided by their path integrator (Knaden and Wehner, 2005). So far, only panoramic landmarks have been subject to experimental studies. These panoramic landmarks are large voluminous objects, exceeding the size of the animal several orders of magnitude and can therefore be detected from quite some distance. But the running ants continuously establish direct contact with the substrate they locomote on. Inspired by the use of tactile cues in spiders (Albert et al., 2001) we wondered if tactile cues monitored by the ant's legs would suffice as navigational cues as well.

2.2 Hypotheses and experiments

In this dissertation I present three studies performed on *Cataglyphis* ants and on the relation between legged locomotion and navigation under severe environmental conditions.

2.2.1 Can mechanical cues serve as landmarks?

The first paper (Seidl and Wehner, 2006; chapter 3) deals with the tactile senses located in the ant's legs and the use of tactile cues for navigation. Desert ants can monitor the tactile properties of the ground it is running on. If, for instance, the surrounding of the nest entrance has tactile and visual properties different from those of the rest of the habitat, the ants will learn these local properties of the ground on which they move and recall them in a similar way they treat purely visual landmarks (Fig. 2.4).

Figure 2.4: Desert ant *C. fortis* entering the artificial nest entrance from the training channel. The black structure is the training landmark characterized by optical and tactile properties.

We trained ants to forage forth and back in a channel and presented them at the nest entrance with a piece of abrasive paper, which differed from the remaining channel floor in both its visual and tactile properties. When the ants were confronted with an identical piece in a separate test channel, they centered their nest search on the nest-defining edge of the landmark rather than on the vector-defined nest position. In order to get accepted

the presented structure had to coincide with that of the learnt ground-landmark both in visual and tactile aspects.

2.2.2 Is active locomotion a prerequisite for path integration?

The second paper (Seidl et al., 2006; chapter 4) focusses on the mechanical prerequisites for path integration. Desert ants need to obtain skylight information in order to integrate steps made (Sommer and Wehner, 2005). Hence we asked whether ants actually have to walk in order to gauge distances. It had been shown previously that in walking desert ants visual flow field cues play only a minor role for distance estimation (Ronacher and Wehner, 1995; Ronacher et al., 2000). The question now was if passively displaced ants were able to integrate the distance covered (like e.g. passively displaced wasps do; Ugolini, 1987), or if they have to walk actively in order to process any distance-information cues. Our study clearly showed that active walking is an absolutely necessary prerequisite for successful path integration (Fig. 2.5).

Figure 2.5: View into the channel prepared for passive displacement of *Cataglyphis* ants. The channel was placed orthogonal to the training setup with an inclination of 53°. The ants were released by the experimenter (left) in the top section of the channel and directly began their uncontrolled and uncoordinated descent (compare Figs. 4.1, 4.2).

2.2.3 How does slope affect the ant's walking behavior?

The third manuscript (Seidl and Wehner, in preparation; chapter 5) of which this thesis is composed consists of two different experiments both aiming at identifying the responsible mechanisms for three-dimensional path integration, i.e. measuring step length and surface inclination.

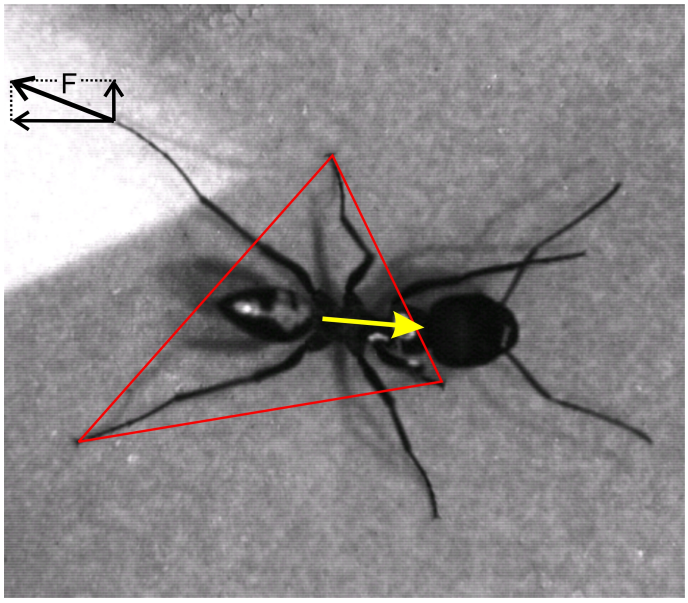

Figure 2.6: Dorsal view of *Cataglyphis fortis* running on an inclined surface. In fast running animals the middle leg and the contralateral front and hind leg move synchronously, forming a biphasic tripod gait (red triangle). During stance phase, the backward swinging leg transmits muscular force (F) to the ground. The backward oriented component of this force generates the forward thrust (yellow arrow) of the animal.

The big question behind this study was: Would the ants change kinematic parameters - both spatial and temporal ones - when running on an inclination? If our high speed cinematographic analysis revealed such changes, i.e. adaptations to surface inclinations, and were these changes correlated with slope, we would be able to pinpoint the location of potential gravity sensors which might provide the ant's path integrator with the necessary signals. Three major possibilities are proposed and discussed on the basis of our results: (i) Geometric changes in the footfall geometry might be used to maintain static stability, but might also be exploited for gravimetry. Sensors monitoring joint positions (e.g. hair field sensors between the joints) could directly provide this information for

monitoring step length and inclination. (ii) The external gravitational force acting on inclined surfaces in or against the desired direction of locomotion influences the temporal patterns of the movements of the legs during the different phases of the stepping cycle. This temporal variation could, for instance, be monitored by hair field sensors that register the time at which the legs reach their anterior and posterior extreme positions. (iii) Even if the externally acting gravitational force and the changing demands for static stability do not influence the kinematic patterns of the ants' locomotion on inclines, there must be changes in the forces acting on the legs, and these changes could be monitored, e.g., by campaniform sensilla within the leg cuticle.

In the first part of the study we performed kinematic analyses of ants running on inclines (Figs. 2.6). This approach provided us with answers to the first two questions raised above. In order to be able to separate species-specific effects from task effects, we compared desert ant locomotion with wood ant locomotion (Fig. 2.7). In the second part of the study, I performed experiments on desert ants foraging on a surface that limited the horizontal thrust producing forces that could be transferred from the animal to the ground. Wind load induced the ants to broaden their step lengths from very short ones to very large ones, depending on the direction of the wind, and hence to break up the relationship between high horizontal forces and long steps, and vice versa (Fig. 2.8).

In summary, the present account shows that ants do not vary their kinematic parameters under a multitude of conditions, so that positional or temporal changes in the ant's locomotory patterns cannot play a role in estimating distances and surface inclinations. Rather, force generation and the direct correlation between force output and step length seem to be involved in accomplishing these tasks. Hence we propose that future experiments should focus on the force sensitive campaniform sensilla within the ant's legs and on their role for three-dimensional path integration on corrugated surfaces.

2.3 General conclusions

Mechanical cues play an important role in desert ant navigation. While celestial compass and panoramic landmark information is taken up and processed by the ant's visual system, further necessary information has to be gained by mechanoreceptive systems.

(a)

(b)

Figure 2.7: Experimental setups for kinematic recordings of (a) *Formica pratensis* in the laboratory and (b) *Cataglyphis fortis* in the field.

The first part of the present PhD-thesis account shows that *Cataglyphis* ants do not only use visual cues for landmark orientation. If the ground around a certain point of interest, e.g. the nest entrance, differs in its tactile cues from the surrounding habitat the ants will learn this tactile feature and later focus their nest search on this nest defining structure. If in an experimental test situation the nest entrance defined by the ground-landmark does not coincide with the nest entrance defined by the path integrator, the ants will

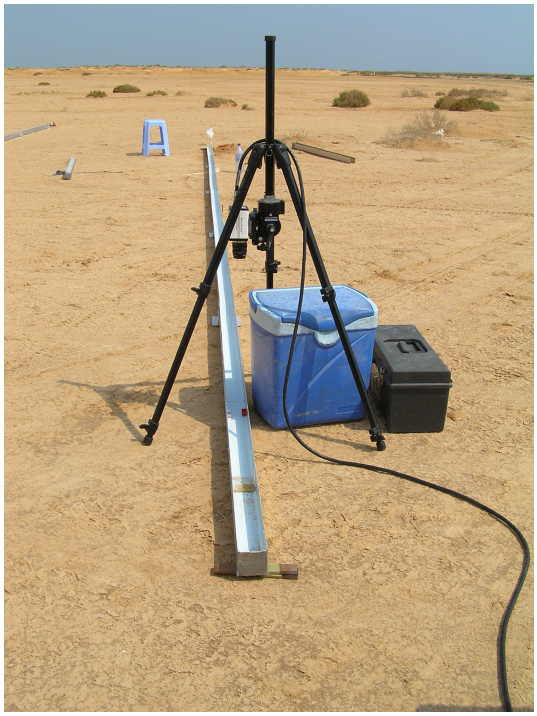

Figure 2.8: Experimental setup for analyzing path integration on slippery substrates. The white ground floor in the training channel is characterized by a surface roughness of 100nm and hence prevents the ants from transmitting high horizontal forces to the ground. The interior of the channel does not provide any visual flow-field cues that could be exploited for distance estimation.

respond to this ground-landmark as they respond to extended panoramic landmarks and focus their nest search on the landmark rather than on the vector-defined position of the nest. In order to recognize a previously learnt ground landmark, the ants have to be presented with both optical and visual cues that are identical with the ones presented during training. One sensory feature alone does not work. As the catchment area of such a small ground structure, i.e. the distance from which it can be detected, is much smaller than that of a large panoramic landmark, the ants will likely loose sight of the ground-landmark and will then have to rely on the information of their path integrator. This effect is similar to the one shown in cases in which panoramic landmarks had been removed temporarily when homing ants were tested (Knaden and Wehner, 2005).

It is also the second part of the present account that focusses on the interplay between visual and mechanical information processing. Previous studies had indicated that distance estimation and slope detection are predominantly performed by idiothetic cues

(Ronacher and Wehner, 1995; Ronacher et al., 2000; Wohlgemuth et al., 2002). However, there was a small but significant influence of visual flow field cues on distance estimation: Flow-field cues in the ant's ventral visual field contributed to about 10 per cent to the ant's odometer, i.e. its distance-gauging device. When in our experiments the ants were prevented from active walking, they completely disregarded any optic flow they could experience.

The third part of the present work aimed at pinpointing the actual sensory mechanisms of slope detection and distance estimation. The results of this cinematographic analysis showed that desert ants employ a very robust walking gait in which the various kinematic parameters do not vary when the animals walk uphill or downhill. These findings exclude hypotheses stating that hair field sensors at the joints of the legs would monitor the working area of the legs or the temporal patterns of the stepping cycle, and that this information would be used to estimate the inclination of the surface on which the animals run. Behavioral experiments with ants running on slippery surfaces induced the ants to break their otherwise rigid relationship between stepping frequency and step length. However, we could at least qualitatively show that as long as step length and horizontally acting forces on the legs coincide, the ants are still able to correctly gauge distances covered. Therefore our findings indicate that monitoring forces along the legs of walking ants might contribute to the ant's abilities of detecting slopes and measuring step lengths.

2.4 Resulting publications and contributions from other scientists

This dissertation contains three chapters based on conceptually and methodically different approaches. Each of these chapters which can be understood in its own, i.e. can be read independently from the other two chapters, represents a manuscript that has already been published (in the first two cases) or that is prepared for submission to a peer reviewed journal (in the third case). In the following I will briefly describe the contents of the three manuscripts and the contributions of other scientists to the work[1]. In addition to the studies presented I have conducted a set of experiments that have not

[1] See page 94 for a complete list of scientific work during my PhD-studies.

been analyzed yet and that will at least partly be published in the future.

2.4.1 Manuscript 1 (Chapter 3)

Tobias Seidl and Rüdiger Wehner (2006). Visual and tactile learning of ground structures in desert ants. *Journal of Experimental Biology* **209**, 3336-3344.

I designed the experimental approach, built the experimental device, performed the experiments during the field seasons 2004 and 2005 (both June to September), and analyzed the data. In discussions with Rüdiger Wehner it became clear that some refinements and additional experiments had to be included. Andrew Martin (University of Applied Sciences, Bremen) kindly provided SEM pictures of the tarsi of *C. fortis*. Ralf Möller and Frank Röben (University of Bielefeld) performed measurements on the optical properties of the landmarks used, and Bernhard Bringmann (ETH Zürich) determined the surface roughness of the ground landmarks used in the experiments. I wrote the first draft of the manuscript, Rüdiger Wehner and I jointly wrote the final version.

2.4.2 Manuscript 2 (Chapter 4)

Tobias Seidl, Markus Knaden and Rüdiger Wehner (2006). Desert ants: is active locomotion a prerequisite for path integration? *Journal of Comparative Physiology A* **192**, 1125-1131.

The basic question and a preliminary setup were developed by me during my field stay at our Tunisian field site during June to September 2004. First results were presented at the Neurobiolgy Conference in Göttingen, 2005 (Seidl and Wehner, 2005). In further discussions Markus Knaden contributed some ideas for technical improvement and an extension of the first experiment. I designed the first and the final experimental setup, performed the experiments (partially supported by Andrew Martin, an intern from the University of Applied Sciences, Bremen, Germany), and analyzed the data. I wrote the first version of the manuscript later annotated by Markus Knaden. The final version was provided by Rüdiger Wehner and myself.

2.4.3 Manuscript 3 (Chapter 5)

Tobias Seidl and Rüdiger Wehner (in preparation). Three-dimensional path integration: how do desert ants monitor slope? *Prepared for submission to the Journal of Experimental Biology*

The kinematic study of ant locomotion was assigned to me by Rüdiger Wehner. In the following I developed the setup for the high-speed recordings and tested this setup on ants (*Cataglyphis noda*) collected during a field trip to Greece (2003) carried out together with Matthias Wittlinger (University of Ulm, Germany), who provided us with a desert proof high-speed video camera. The camera was financed by the Volkswagen Stiftung in a grant assigned to Reinhard Blickhan (Friedrich Schiller University, Jena, Germany), Bernhard Ronacher (speaker; Humboldt University, Berlin, Germany), Rüdiger Wehner (University of Zurich), and Harald Wolf (University of Ulm, Germany).

Figure 2.9: Preliminary experiments with *Cataglyphis noda* on the roofs of Irchel Campus buildings, University of Zurich.

In the following we established a cooperation with the biophysics laboratory of Reinhard Blickhan and extended the study on desert ant-locomotion to a comparable one on wood ant-locomotion (*Formica pratensis*). The indoor cinematographic recordings were done in Jena during the time period October and November 2003 (Fig. 2.7a). The recordings involving dorsal and lateral views of the running, channel-bound ants were

done in cooperation with Tom Weihmann (Jena) and tracked by student helpers in the laboratory of Reinhard Blickhan. While I analyzed the entire dorsal-view data, Tom Weihmann analyzed the lateral-view data. For my analysis I wrote Matlab-routines in order to import and process the data. In Jena, Tom Weihmann gave me an introduction to Matlab programming. In the following I analyzed the results statistically using the software package R (www.r-project.org), with helpful hints on statistical procedures from Lorenz Gygax (Agroscope FAT - Eidgenössische Forschungsanstalt für Agrarwirtschaft und Landtechnik, Tänikon) and Wolf Blanckenhorn (University of Zürich).

The experiments on path integration on a slippery surface was an idea of my own and was developed further in discussions with Rüdiger Wehner and Walter Federle (Cambridge University, UK). I designed both the preliminary and the final setup, performed the experiments (partially supported by the intern Andrew Martin, already mentioned above) and analyzed the data. I wrote the first version of the manuscript later revised by Rüdiger Wehner and jointly put into its present form with him.

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3 Visual and tactile learning of ground structures

Tobias Seidl and Rüdiger Wehner (2006). Visual and tactile learning of ground structures in desert ants. *Journal of Experimental Biology* **209**, 3336-3344.

Abstract Place defining landmarks that have been studied intensively in insect navigation are large, voluminous objects visible to the insect from quite some distance. Here we show that in desert ants, *Cataglyphis fortis*, local variations in ground properties can also serve as landmarks. The ants were trained to forage within a linear channel, in which the floor adjacent to the nest entrance was altered in optical and tactile properties. When ants were later tested within a test channel that ran parallel to the training channel, they recognized this landmark and centered their search on the part of the ground structure that during training was closest to the nest entrance. Hence, physical properties of the ground can be learnt and used as cues defining, e.g., the position of the nest. In a second series of experiments the ants were presented with ground structures that differed in their visual and tactile properties from the training structure. We show that the absence of either the correct tactile properties or the correct optical properties of the ground structure make the ants reject the previously accepted structure. Hence small ground structures are recognized by the ants as familiar landmarks only if both visual and tactile information coincides with what the ants have experience during training.

Key words *Cataglyphis fortis*, orientation, ground landmark, tactile learning

3.1 Introduction

Desert ants of the genus *Cataglyphis* live in wide, featureless salt-pan areas that provide almost no terrestrial orientation cues. Due to the sparsely distributed food sources (i.e. dead insects that have succumbed to the environmental stress conditions) and the high temperatures the ants have to perform wide ranging individual foraging journeys and do not recruit by, for instance, laying odor trails. Path integration serves as their main means of navigation (e.g. Wehner, 1992; Collett and Collett, 2000; Wehner and Srinivasan, 2003; Wehner et al., 2002). This mode of navigation relies on acquiring egocentric information on the nest position by continuously integrating the path run by the animal, and doing so by integrating directional information provided by celestial cues and distance information provided, e.g., by proprioceptive cues. The outcome of this computational process enables the ant to return to the nest without detour once an appropriate food item has been found. However, due to the egocentric nature of the path integration process the position of the nest entrance as defined by the path integrator (in the following referred to as the PI-position) is subject to a continuous accumulation of errors. In trying to eliminate this navigational uncertainty, desert ants will - whenever possible - take advantage of landmark information, in order to finally pinpoint the goal, the nest entrance, by recalling a previously learnt panoramic image of the goal and comparing this reference image with the current retinal image (Wehner and Räber, 1979; Cartwright and Collett, 1983; Wehner et al., 1996; Wehner, 2003). A basic set-up for testing the significance of landmark navigation with respect to path integration consists in presenting the nest defining landmarks in a position (in the following referred to as the LM-position) that does not coincide with the PI-position (e.g. Wehner et al., 1996; Knaden and Wehner, 2005).

The landmarks used so far in this kind of cue competition experiment have mostly been black cylindrical objects up to 50 cm high and 50 cm wide, i.e. landmarks that have been several orders of magnitude larger than the animal itself, but have mimicked the salt-bush vegetation at the borders of, and sometimes within, the salt pans inhabited by *Cataglyphis fortis* (Heusser and Wehner, 2001). Due to their sheer size these landmarks could be recognized by the ants from quite some distance. The area within which an animal can identify such landmarks and use them for orientation is called the catchment area of the landmark(s) in question (Cartwright and Collett, 1983); hence, the larger the

object, the wider its catchment area.

In addition, there are already a few hints that ants can recognize minor ground variations and use them during nest finding behavior (Santschi, 1913, Fig. 4; Wehner, 1968, and unpublished observations). Inspired by these episodic remarks we designed an experimental setup that enabled us to test whether local variations in the ground properties perceived by the ant's ventral retina can be learnt, recognized and used as landmarks in a potentially similar way as *Cataglyphis fortis* has been shown to use large, panoramic landmarks that extend upwards from the ant's visual horizon. We further tested whether ground structures can be perceived also by tactile rather than only by visual means.

In a first set of experiments we trained ants to a specific ground structure close to the location of the nest, and later presented this structure in a test situation, in which the LM-position differed from the PI-position. Due to the flat geometry of such ground landmarks ants will cross them quite easily. Therefore the ants might be able to use sensory cues other than visual ones, e.g. tactile ones, for recognizing these ground landmarks. It is well known that in the tarsi of arthropods cuticular mechanoreceptors reach a high level of sensitivity and perceptual sophistication (crickets: Gnatzy and Hustert, 1989; flies: Seifert and Heinzeller, 1989; spiders: Albert et al., 2001). However, apart from moths, *Manduca sexta*, which use mechanoreceptors on their proboscis to locate nectar sources by exploiting tactile properties of flower petals (Goyret and Raguso, 2006), there have been no studies published yet which demonstrate the use of tactile cues in any navigational tasks. Hence in a second set of experiments we focussed on the type of stimuli used by the ants in recognizing the ground landmark. Again, the experiments were designed in such a way that the LM-position and the PI-position were set into competition, but now the test landmarks differed from the training landmarks either in their visual or tactile properties.

3.2 Experiment 1

In the first set of experiments we test whether ground landmarks can be used by ants at all and, if they can, how they compete with the ant's path-integration system. Answering the latter question will enable us to place the importance of ground structures into the

broader context of the ant's navigational toolkit. For example, it will enable us to compare the significance of these ground structures with the one of large, panoramic landmarks, i.e. landmarks extending above the ant's visual horizon. Until now it has been only the latter type of landmark that has been studied in *Cataglyphis* navigation (e.g. Wehner and Räber, 1979; Wehner et al., 1996; Åkesson and Wehner, 2002; Bisch-Knaden and Wehner, 2003), and that has been set into competition with the ant's path-integration system (Bregy and Wehner, 2003; Knaden and Wehner, 2005).

3.2.1 Material and Methods 1

All experiments (i.e. experiments 1 and 2) were performed with wild, free-ranging salt-pan ants, *Cataglyphis fortis* Forel 1902 (Wehner, 1983), at our Maharès study site (southern Tunisia) during the months July to September of the years 2004 and 2005.

Ants (nest coordinates: N 43° 31.720' / E 10° 32.278') were trained to forage to a biscuit-crumb feeder located 9m north of their nest. While foraging the ants had to run back and forth between nest and feeder within a linear channel (dimensions: height: 9cm, width: 7cm; material of walls: plywood boards, ground: natural salt-pan area; Fig. 3.1), which they were not able to leave by themselves. During landmark training a piece of dark and very rough abrasive paper (1m long, 7cm wide) was placed on the ground of the channel as close to the nest entrance as possible (for detailed training situations see Tab. 3.1, column 3; Fig. 3.2). Due to this experimental set-up the foraging ants were forced to cross this piece of abrasive paper and by this to experience optical and tactile cues that differed from the ones in the remainder of the channel. Furthermore the channel walls inhibited the ants from lateral view of the surroundings and thus prevented them from seeing additional natural landmarks potentially occurring in the vicinity of the experimental device.

Ants arriving at the feeder were marked with a day-specific color code and tested on the following day or one or two days later. This training-and-test schedule ensured that the ants had completed about 30 foraging round trips, before they were tested (Åkesson and Wehner, 2002). In the critical tests the ants were captured at the feeder and, while still carrying their food item, transferred to a separate test channel (length: 18m) running parallel to the training channel. The test channel was free of foraging

nest mates and food items. This procedure assured that homebound ants searching for their nest could only rely on within-channel landmarks or on their egocentric vector information. Depending on the experimental paradigm the tested ants were confronted with an identical reproduction of the ground landmark used during training, but presented at varying positions relative to the ants' point of release (tests 1A-1E, Tab.1). Once the ants had run off their home vector, they would start their systematic searches centered on the point where they expected the nest to be (Wehner and Srinivasan, 1981). Within the linear test channel this kind of behavior usually performed in two-dimensional space is restricted to one dimension. Constrained by the channel walls the back-and-forth running ants have to execute sharp turns of direction (Sommer and Wehner, 2004). During this 'linear search' six turning points were recorded with a precision of 0.1m. A turn was considered complete if the ant continued to run in the new direction for at least 0.2m. Each ant was tested only once throughout the whole experiment. Tests in which the ants lost their food item or left the test channel before completing the required number of six turns or did not approach the landmark for 0.5m were considered unsuccessful and therefore excluded from the analysis.

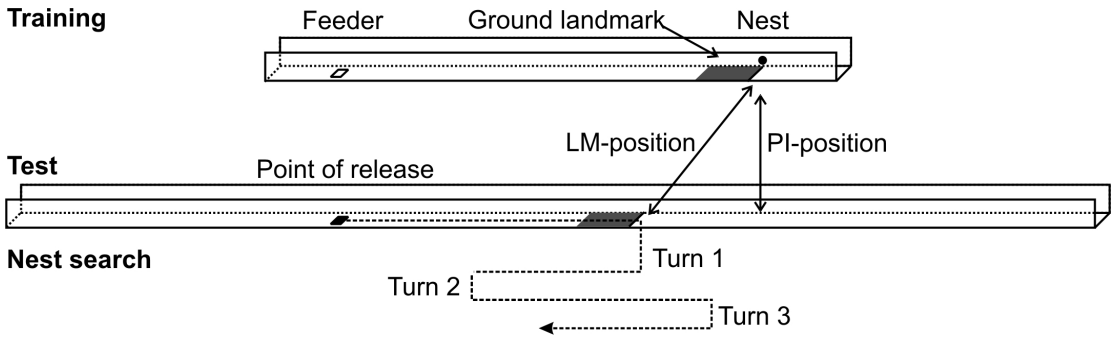

Figure 3.1: Schematic view of the experimental setup. Training took place in a 10m channel with an exit hole on the side, where ants were able to enter and leave the setup and forage to a feeder 9m down the channel. The dark area denotes the position of the ground landmark during training (PI-position). For tests the ants were transferred into another channel 18m long and aligned parallel to the training channel. Within this test channel ants were presented with a ground landmark at varying positions (LM-position) relative to the position indicated by their path integrator (PI-position, experiment 1), or with ground landmarks differing from the training landmark in visual or tactile properties (experiment 2). The first six U-turns of the ant's search behavior were recorded. Drawings are not to scale.

Data analysis

As already mentioned an ant will change its behavior after it has run off its home vector in a straight path, and will search for the nest by systematic back-and-forth movements centered on the fictive position of the nest entrance. This behavior allows us to calculate search density distributions. In order to determine the search density distribution in each individual ant, we divided the channel in virtual 0.1-m bins and calculated how often each bin was visited by the ant. For normalization we then divided the number of visits within each bin by the total number of visits within all bins.

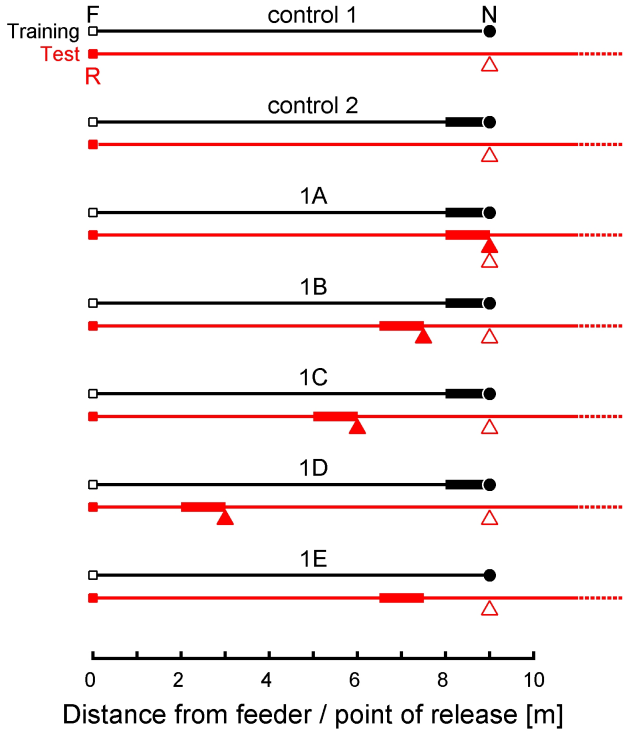

Figure 3.2: Schematic representation of the position of the nest (N) as defined by the path integrator (PI-position, open arrow-head) and as defined by the ground landmark (LM-position, filled arrow) in the different sub-sets of experiment 1. During training both positions coincide, but in the test situations the LM-position is usually shifted away from the PI-position towards the point of release (R). This decoupling of PI-position and LM-position ensured that within the test channel the ants encounter the landmark before they have run off their home vector, i.e. before they have reached the PI-position. F and open square: feeder in the training channel, filled square: point of release in the test channel, N and filled circle: nest in the training channel, black bar: ground landmark.

Search densities of different test paradigms were compared to those of control groups ('control 1' or 'control 2'; for details see: Tab. 3.1, column 7) by Mann-Whitney U-tests (Mann and Whitney, 1947). The analysis focussed on those sectors of the compared data sets that included relevant points ('test area', see column 5 in Tab. 3.1), e.g. the nest position as defined by the landmark (LM-position) or the nest position as defined by the ant's path integrator (PI-position).

3.2.2 Results 1

3.2.3 Do ants use ground landmarks?

The first set of experiments aimed at answering the question whether *Cataglyphis* ants, once transferred to the test channel, focussed their nest search behavior more sharply on the fictive position of the nest if the ground landmark were present than if it were not. This was indeed the case (Fig. 3.3). When ants were trained with a landmark at the nest and confronted with the identical set-up in the test channel, i.e. with the LM-position coinciding with the PI-position, (Fig. 3.3: test 1A), the ants searched mainly and consistently at the fictive position of the nest as defined by both the landmark and the state of the path integrator. The height and sharpness of the search peak significantly differed from that of ants that had been trained and tested without any landmark (Fig. 3.3: control 1) or that had been trained with the landmark at the nest position, but had later been tested without it (Fig. 3.3: control 2). In fact, the ants' search behavior did not significantly differ between both types of control (Tab. 3.1, last column, first row), but did so highly significantly between 'control 2' and test '1A' (Tab. 3.1, last column, third row). Hence, ground structures such as the ones used in the current set of experiments are effectively used by the ants as signposts marking the nest entrance.

3.2.4 Displaced ground landmarks and nest search.

In the next set of experiments the ground landmark was set in competition with the ant's path integrator. In technical terms, LM-position and the PI-position did not coincide. This was achieved by presenting the ants with the landmark at a location at which they

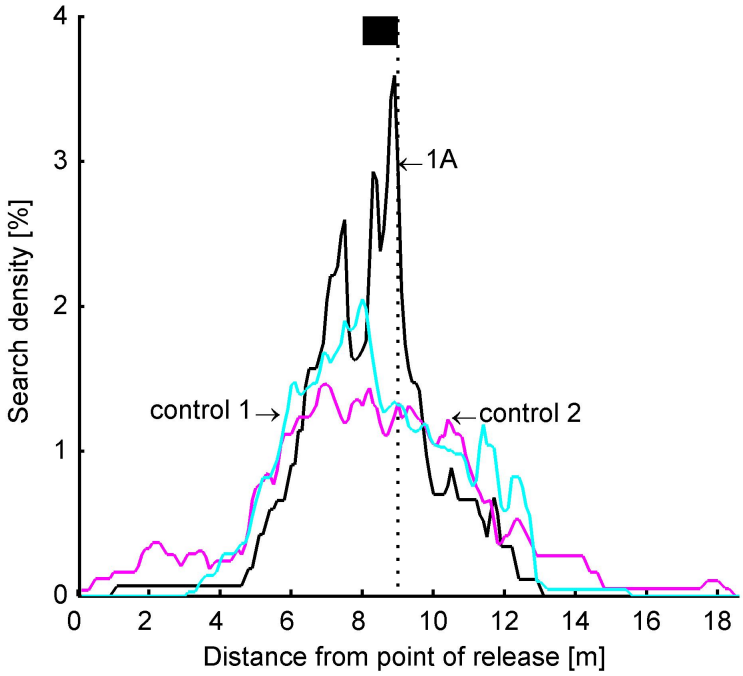

Figure 3.3: Search density distribution of the ants' nest search behavior exhibited under different training and test conditions. Control 1. Ants were trained and tested without any landmark. Control 2. Ants were trained with a landmark located at the nest entrance but tested without one. 1A. Ants were trained and tested with a landmark at the nest entrance. (Black square: position of landmark; dotted line: position of path-integrator-defined nest.)

had not yet run off their home vector, so that they had to decide between the PI-position and the LM-position of their goal (Fig. 3.4). As a control we used ants in which the landmarks present during training had been removed in the test situation, so that the ants had to rely exclusively on their path integrator (Fig. 3.4a, control 2).

If the LM-position differed from the PI-position by 1.5m (Fig. 3.4a: test 1B) the search density at the LM-position (7.5m) was significantly higher than that at the corresponding position of the control animals. Actually, it was as high as the one obtained when the LM-position coincided with the PI-position (Fig. 3.4a: test 1A). If the LM-position was moved even closer to the ants' point of release, i.e. even further away from the PI-position (3m; Fig. 3.4a: test 1C), the ants still focussed their search at the LM-position (6m) highly significantly more strongly than the control animals did. However, if the ants encountered their nest landmark very close to the point of their release, i.e. if the LM-position differed from the PI-position by 7m (Fig. 3.4a: test 1D), the ants did no

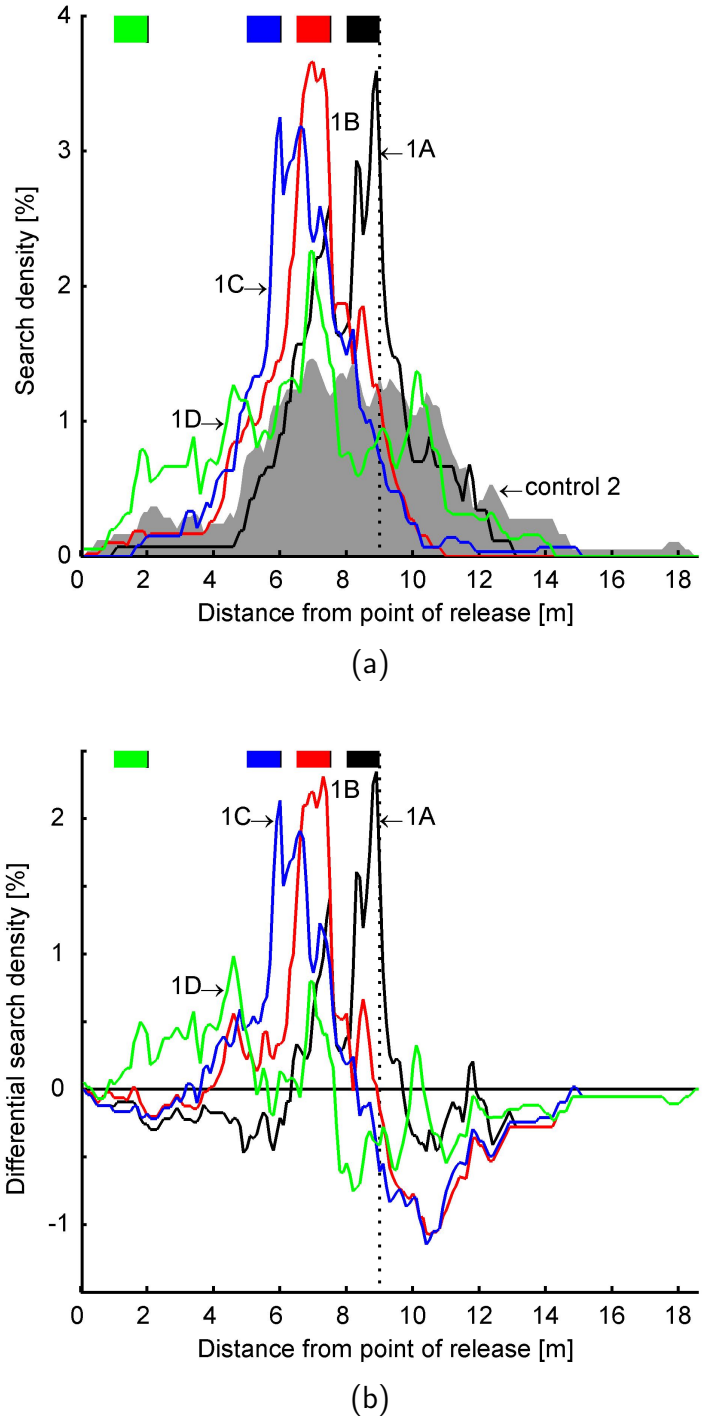

Figure 3.4: A. Search density distribution and B. differential search density distribution of the ants' nest search behavior exhibited under different test conditions. All ants were trained with a landmark (black square) directly by the nest and tested with a displaced landmark (colored square). 1A. Control: LM-position (identical to the one in the training situation): 9m (black line and square). 1B. LM-position: 7.5m (red line and square). 1C. LM-position: 6m (blue line and square). 1D. LM-position: 2m (green line and square). Control 2. no landmark present during test. Note: in Fig. 3.4b the data of control 2 (Fig. 3.4a) were subtracted from each other data set for display reasons.

longer use the landmark as an orientational cue. Their search density at the LM-position (3m) did not differ from the corresponding one in the control 2. Mean values, standard deviations, and p-values of all statistical tests mentioned in the paragraph above are given in the last three columns of Tab. 3.1.

3.2.5 Effect of unknown ground structures

As shown above, ants do use ground landmarks for orientation. But how do they deal with a structure on the ground which they have never encountered before? If ants were trained without a landmark but were presented with one during the test (Fig. 3.2: test 1E), their searches exhibited a prominent peak in front of the landmark and far away from both the LM-position and the PI-position (Fig. 3.5). Their behavior was significantly different from that of naïve ants that did not encounter a landmark during both training and testing (Fig. 3.3: control 1; Tab. 3.1, last column, last line). In contrast to the latter control situation, the ants rigorously tried to avoid the landmark, and as they could not leave the channel, they ran back and forth without crossing the landmark and hence never got close to the PI-position.

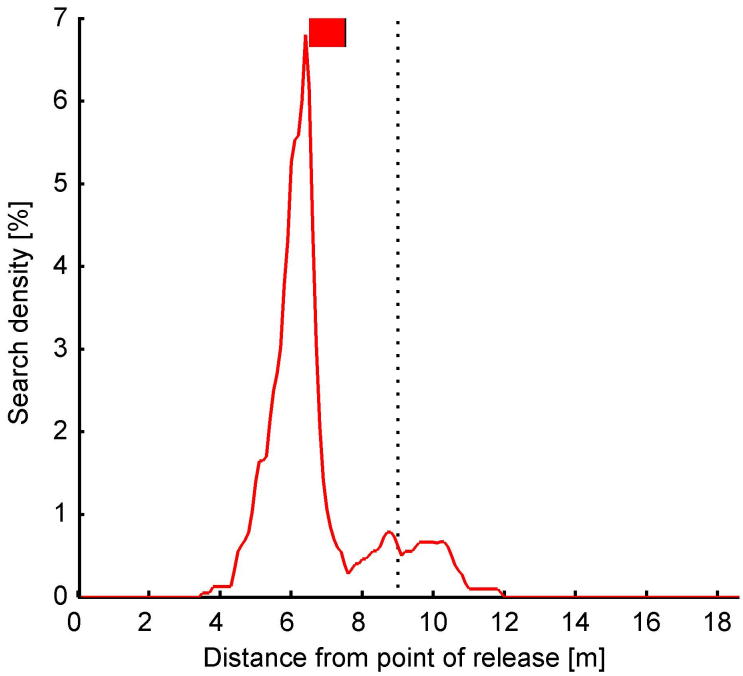

Figure 3.5: Search density distribution of ants that faced a landmark for the first time (1E). Ants were trained without a landmark, but were presented with a landmark (LM-position: 7.5m) in the test situation.

Table 3.1: Overview of experimental subsets of setup 1. All used landmarks were of dark color with a rough surface. The rear boarder of the 1-m landmark defines the LM-position. For each experiment a certain area of interest was determined and compared statistically to the data of the same area from a control group using the U-test.

Experiment	n	Rear boarder of landmark [m]		Test area [m]	Search densities in test area[1]			U-test
		Training	Test		Experimental group [%]		Control group[2] [%]	p-value
control 1	20	-	-	8.5-9	1.31±1.03	*vs.*	1.20±0.95	0.66
control 2	20	9	-	8.5-9	1.20±0.95	-	-	-
1A	15	9	9	8.5-9	2.96±2.33	*vs.*	1.20±0.95	*<0.01*
1B	20	9	7.5	7-7.5	2.70±1.59	*vs.*	1.32±1.09	*<0.01*
1C	20	9	6	5.5-6	2.58±1.70	*vs.*	1.10±1.10	*<0.01*
1D	20	9	2	1.5-2	0.61±1.10	*vs.*	0.29±0.82	0.13
1E	20	-	7.5	8.5-9	0.71±1.35	*vs.*	1.31±1.03[3]	*<0.03*

[1]) All values given are mean±s.d. [2]) The experiments' search densities at the test area specified in column 5 were tested against the analogue data section from 'control 2' except [3]) Control group: 'control 1'.

3.3 Experiment 2

In contrast to panoramic visual landmarks which can be perceived from a distance, ground landmarks can be detected only when the ant walks over them. In doing so, the ants come into direct contact with the ground structure not only visually, but also mechanoreceptively. Therefore we examined whether the ants were able to exploit this tactile input as well. In particular we ask whether the ants can detect mechanical properties of the ground structure, such as the roughness of the surface, and use them for orientation.

3.3.1 Material and Methods 2

In the second set of experiments the ground landmark was altered stepwise in both its optical and its tactile properties: All ants were trained with a black and rough landmark delivering high visual and tactile contrast to the channel surroundings. Later they were tested with landmarks differing in their optical and tactile properties and having been displaced from the PI-position towards the point at which the ants had been released, by 1.5m (LM-position: 7.5m; Fig. 3.7).

The ants (nest coordinates: N 34° 31.745' / E 10° 32.333') again had to forage for 9m within a north-south oriented channel (dimensions: height and width: 7cm each, material: walls and ground: aluminium) to a feeder. The original nest entrance was connected to the channel by a thin tube. The interior of the channel was spray-painted grey (for optical properties see Fig. 3.6) with the ground additionally being covered with light brown sand. The training landmark consisted of the abrasive paper mentioned above, but it was now spray-painted black in order to increase the visual contrast between structure and ground. The use of the identical paint on the different landmark materials (abrasive paper, cardboard) also eliminated possibly different olfactory cues emerging from the different structures. During training the landmark was again placed at the nest entrance (Fig. 3.7, LM-position and PI-position: 9m), whereas in the tests its position was slightly moved towards the point of release (LM-position: 7.5m; compare Fig. 3.2: test 1B).

In analyzing the type of stimuli being used by the ants, in the test situation different

types of landmarks were applied (see Tab. 3.2, columns 3 and 4 for landmark description and Fig. 3.6 for their optical properties): a black and rough landmark identical to the training landmark (test 2A); a landmark painted in the background color of the channel but identical in tactile cues with the training landmark (test 2B); and finally a black-sprayed piece of cardboard providing the same optic but different tactile cues as the training landmark (test 2C). In a further experiment we covered the lower hemispheres of the ants' eyes by applying light-tight acrylic paint (after the ants had been trained) and put the ants back into the nest (test 2D). Once the treated ants had reappeared at the feeder, we provided them in the test situation with a grey and rough landmark. Now the ants did not experience any visual cues - not even diffuse ones - that could indicate the position of the ground structure (Antonsen and Wehner, 1995). After a successful test (see above) each ant was examined under a binocular microscope to confirm that the coverage was still intact. Due to this post experimental test 5 of the 17 treated ants had to be excluded. In each ant the search behavior was recorded and the search density was computed by following the same protocol as in experiment 1. The search density profiles of the four experimental subgroups mentioned above were compared by applying the Kruskal-Wallis-test (Kruskal and Wallis, 1952) accompanied by the Dunn-Sidak Multiple Comparisons post test (Hochberg and Tamhane, 1987).

3.3.2 Results 2

3.3.3 Relevant stimuli for ground landmark recognition

In the control experiment (test 2A), in which the optical and tactile properties of the landmark coincided with the ones during training, the ants searched consistently at the LM-position. However if either parameter of the landmark was changed (optical properties: test 2B, tactile properties: test 2C), the ants behaved similarly to the ones in experiment 1 in which the ants had never seen a landmark during training but were presented with one in the test (Fig. 3.5: test 1E): They avoided trespassing the altered landmark. This behavior resulted in a clear peak positioned directly in front of the landmark, far away from both the LM-position and the PI-position (Fig. 3.7: tests 2B and 2C).

However, the search density distribution of the ants confronted with the invisible landmark (test 2B) was bimodal exhibiting a second peak at the PI-position. If the ants had once happened to cross the landmark, they avoided the LM-position and searched at the PI-position. This bimodal distribution with maxima in front of the landmark and at the PI-position could also be observed, if the ants prior to testing had been deprived of vision in their ventral field of view by covering the lower hemispheres of their eyes with light-tight paint (Fig. 3.7: test 2D). Even though the half-blinded animals subsequently encountered a familiar tactile landmark, they nonetheless avoided trespassing it. The results of statistical tests are given in Table 3.2.

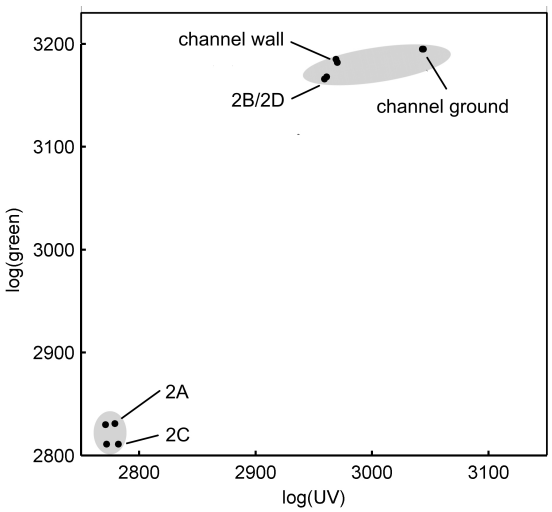

Figure 3.6: Visual properties of the landmarks used during experiment 2. The optical properties have been determined following Kollmeier (2005) by measuring the remission properties under natural (sunlight) conditions at the specific wavelengths of the ant's light receptors (absorption maxima: green: 500nm, bandwidth: 90nm; UV: 350nm, bandwidth: 60nm). The optical properties of the landmarks are predominantly defined by the paint used and not by the surface roughness.

3.4 General Discussion

3.4.1 Ants perceive and use ground landmarks

Our experiments show that marginal variations in ground properties do play a role during an ant's search for the nest. In their natural environment ants can use such local structures to memorize and later recall minute characteristics of the ground surface in the immediate vicinity of the nest entrance. Similarly to what happens in the presence of large panoramic visual landmarks defining the position of the nest (Wehner et al., 1996; Bregy and Wehner, 2003), small ground structures are used as nest-defining landmarks

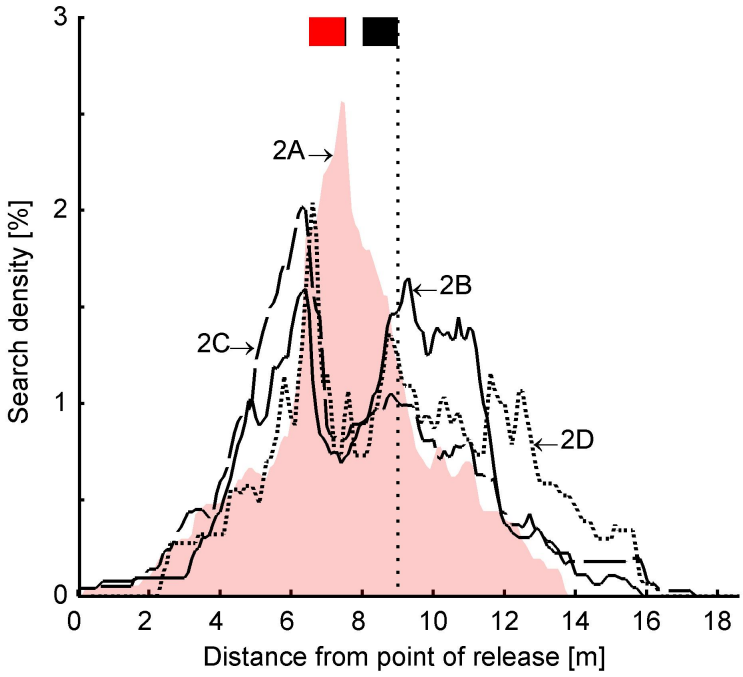

Figure 3.7: Search density distribution of ants that faced different types of landmark stimuli. Ants were trained with a black and rough landmark at the nest entrance (black square) and later tested with a landmark of different properties defining the nest entrance at 7.5m (red square, LM-position). 2A. Control experiment with a black and rough landmark (same experimental situation as experiment 1B, but new data set). 2B (thin solid line). A grey and rough landmark deprived the ant of the visual contrast. 2C (dashed line). A black and smooth landmark changed the surface roughness, but left the visual contrast intact (as compared to the training situation). 2D (dotted line). The lower hemispheres of the ants' eyes were covered with light tight paint depriving the ant from any visual cues from below.

only within certain states of the ant's path-integration vector: If an ant has learnt a nest landmark, later during homing it expects this landmark to occur after it has fully run off its home vector. But as all vector navigation is error prone, landmarks of any kind will help in finally pinpointing the goal. Particularly, ants might accept a landmark at different states of their home vector, i.e. already after they have not yet completed their vector-based home run. In the present study the ground landmark was accepted by the ant as a nest-defining orientation cue at vector states of 100%, 83% and 66% (Tab. 3.1: series 1A-1C), but it was ignored at the 22% state (Tab. 3.1: series 1D). This result is very similar indeed, even in quantitative terms, with the results obtained by using large panoramic landmarks as nest defining cues (Bregy and Wehner, 2003).

However, the influence a familiar ground landmark has on the ant's search pattern is limited: The center of search cannot be shifted by the ground landmark as drastically away from a position defined by the ant's path integrator as is the case with panoramic landmarks (e.g. Knaden and Wehner, 2005). This can easily be explained by the reduced catchment area of ground marks: Whereas large panoramic landmarks can be seen from far away, a ground landmark can be recognized by the ant's ventral field of view and by the mechanoreceptors of the ant's tarsi only if the ant is directly above it. If the ant leaves the structure and loses it not only tactilely, but also visually, it instead continues to rely on its path-integration vector. Knaden and Wehner (2005) have shown a similar effect with panoramic visual landmarks: If in the test field a set of nest-defining

Table 3.2: Overview of experimental subsets of setup 2. Throughout the whole experiment the ants faced a black and rough landmark in position 9 during training. For tests landmark properties were altered but all landmarks were presented in LM-position = 7.5m, i.e. with the rear end of the landmark at 7.5m. Mean search densities of all experiments at the rear end of the test landmark (Test section: 7-7.5m) were compared statistically. Different letters (last column) indicate significant difference at α=5%.

Experiment	n	Landmark properties		Search densities at rear end of landmark[1] [%]	Kruskal-Wallis test with Dunn post test	
		visual	tactile		p-value[2]	group
2A	24	black	rough	2.38±1.47	-	**a**
2B	25	grey	rough	0.74±0.61	*<0.001*	**b**
2C	19	black	smooth	0.83±0.68	*<0.01*	**b**
2D	12	invisible	rough	0.89±0.99	*<0.05*	**b**

[1]) All values given are mean±s.d.. [2]) p-values for pair-wise comparison against 2A.

cylindrical landmarks was placed at a position that differed from the position defined by the ant's path integrator, i.e. if LM-position and PI-position did not coincide, the ants first searched, unsuccessfully of course, at the PI-position and - after the landmarks had been installed - switched to the LM-position, but after removal of the landmarks by the experimenter returned immediately to the PI-position. This shows that the path integrator keeps running all the time, even if the ants happen to rely, at a particular instance, on landmark information.

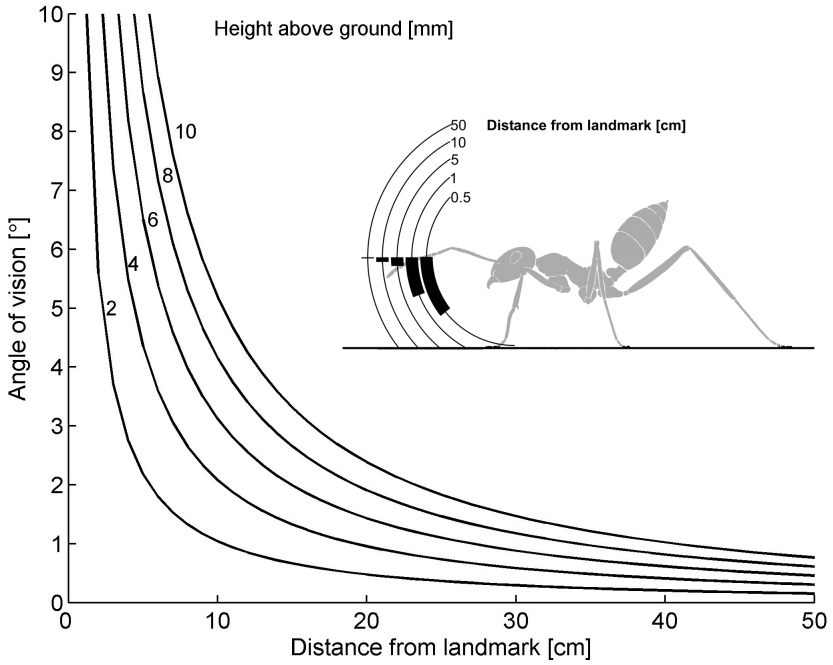

Figure 3.8: Ants perceive a ground landmark only from a short distance. Main figure. The angle of vision under which the ground landmark (1 m, 0.07m) appears in the ant's visual field depends strongly on the distance of the ant from the landmark (shown here for distances $<$0.5m). The height of the eye above ground (2 – 10mm) has a minor effect. Insert figure. Vertical expansion of the ground landmark used in the current experiments within the ant's visual field at different distances of the ant from the landmark (eye 4mm above ground). The landmark remains extremely small ($<1^\circ$) up to an approach of about 20cm. Then it rapidly expands covering a large part of the ant's ventral visual field.

3.4.2 Visual and tactile properties play a role

As to the visual perception of the ground structures we can deduce from our field observations, i.e. from short stops or unrecorded turns performed by the ants close to

the landmark border, that within the test channels the ants can most certainly recognize the landmarks visually from a distance of about 10-15cm. We calculated the angular subtense of the landmark within the ant's visual field of view for various distances between ant and landmark, and for various heights of the ant's eyes above ground (Fig. 3.8). The calculations show that the landmark remains small within the ant's field of view until the ant has approached it for a distance of about 0.2m. From that distance onwards the retinal image of the landmark increases dramatically in size until it covers almost the entire ventral visual field (e.g. lateral expansion: 166°, ventral expansion: 188° if the ant's eye is located 4 mm above ground).

The importance of the tactile properties of a ground landmark could be shown most directly in the experiments in which the ventral halves of the ant's eyes were covered with light-tight paint, so that any visual input from the ground structure was abolished. Yet the ants, if forced to perform their home run in the test channel, avoided a novel ground mark. Hence mechanosensory information alone suffices in detecting ground structures. But visual information alone does so as well. In fact, whenever in the test situation the ground landmarks were changed relative to the training situation in either their optical or their tactile properties, the ants exhibited an avoidance response (Fig. 3.7: series 2B and 2C). Obviously, in either case the landmarks were no longer recognized as the ones with which the ants had been familiar in the training situation.

But how exactly did the animals perceive the tactile information provided by the ground structures offered in the present case? The structures used in this study most obviously differed in surface roughness, i.e. in the height and the frequency of the deposited grains. As a qualitative analysis of numerous high-speed video recordings (e.g. Seidl et al., 2004) did not provide any hint that the ants used their antennae for evaluating surface structures during locomotion, the tarsi are the most likely candidates for analyzing the geometric surface properties. If we compare the geometry of an ant's tarsi with the surface profiles of the ground structures used in this study (Fig. 3.9), we find that on the sanded channel ground the ant's tarsi would come to lie on top of a layer of densely deposited grains, while in the case of the abrasive paper they would fit in between the gaps of the loosely spread substructures of the paper. Tactile hairs on the tarsi could determine the geometry of the surface structure by monitoring both grain height and deposition frequency. Another way of how the ants could monitor surface structures would be to exploit surface elasticity by, e.g., campaniform sensilla within the cuticle

or muscular strain sensors during touch down or lift off of the tarsi. Even though mechanoreception is a well known sensory capacity in insects (e.g. Römer, 2003), most studies deal with mechanoreceptors located at the antennae (e.g. Martin and Lindauer, 1966). Tarsal mechanoreceptors have so far been studied in spiders (e.g. Foelix, 1970), but not yet in insects.

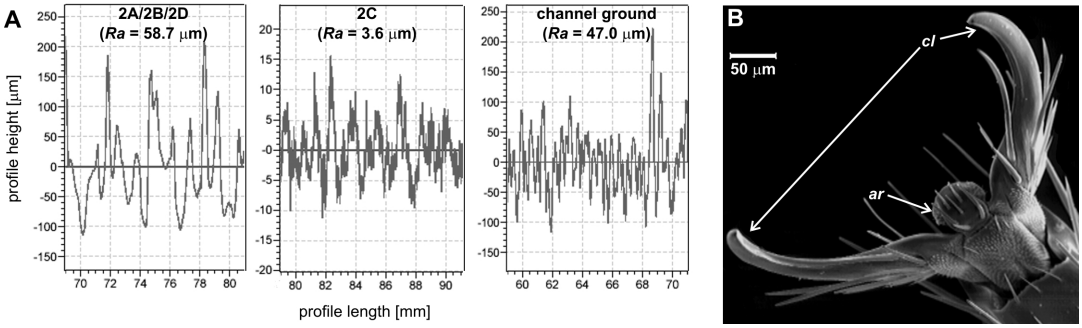

Figure 3.9: A. Typical surface profiles of the structures used in experiment 2 determined using a contact-profilometer. The roughness is characterized by Ra, defined as the arithmetic mean of the deviations from the base line measured over an evaluation length (DIN EN ISO 4287). The mean diameter of the grains deposited on the abrasive paper used for experiments 2A, 2B, and 2D (compare Tab. 3.1) is 270μm, the sand glued to the channel ground had a mean diameter of 200μm. In contrast to the abrasive paper the sand grains are deposited next to each other without gaps. B. Dorsal view of a tarsus of *Cataglyphis fortis* (ar: Arolium; cl: Tarsal claws). Tarsal claws are separated from each other by approximately 320μm. (SEM micrograph: Andrew Martin)

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4 Is active locomotion a prerequisite for path integration?

Tobias Seidl, Markus Knaden and Rüdiger Wehner (2006). Desert ants: is active locomotion a prerequisite for path integration? *Journal of Comparative Physiology A* **192**, 1125-1131.

Key words *Cataglyphis fortis*, locomotion, path integration, optic flow, proprioception

Abstract Desert ants *Cataglyphis fortis* have been shown to be able to employ two mechanisms of distance estimation: exploiting both optic flow and proprioceptive information. This study aims at understanding possible interactions between the two possibly redundant mechanisms of distance estimation. We ask whether in *Cataglyphis* the obviously minor contribution of optic flow would increase or even take over completely, if the ants were deprived of reliable proprioceptive information. In various experimental paradigms ants were subjected to passive horizontal displacements during which they perceived optic flow, but were prohibited from active locomotion. The results show that in desert ants active locomotion is essential for providing the ants' odometer and hence its path integrator with the necessary information.

4.1 Introduction

During their foraging excursions desert ants of the genus *Cataglyphis* are confronted with a uniform environment that does not necessarily provide sufficient external orientation

cues. Therefore these ants rely on path integration (vector navigation) as their main means of navigation. As this strategy demands egocentric information on the animal's actual position, the home vector must be updated continually by integrating the segments of a foraging path and hence determining both direction and length of each single segment.

While the ant's walking direction is deduced from skylight cues (Wehner, 2003), distance estimation seems to be based on two mechanisms: optic flow as perceived within the ventral visual fields of the eyes plays a certain role, but is dominated by additional, potentially proprioceptive, information (Ronacher and Wehner, 1995). The significance of the optic flow was determined in ants that were walking on a transparent ground-floor with a moving pattern underneath. Hence the walking animals had the possibility to at least partially compensate the insufficiencies of the optic flow system by using the correct input from the proprioceptive system, e.g. a step counter as proposed by Wittlinger et al. (2006). However, how do the ants integrate optic flow information into their path integrator, if the inputs from both systems do not coincide? Does one system take over if the other fails to work properly, or does the odometer compute a mean value of both inputs? In addressing this question we disentangled the two systems completely by letting the ants experience passive displacements during which they could not walk properly, but were able to experience optic flow cues.

4.2 Material and methods

In order to have sufficient control on the ants active and passive movement trajectories, and at the same time controlling the presence of optic flow cues we enclosed the ants' movements in channels. All former channel experiments performed with *Cataglyphis* ants used test channels which had been visually as homogenous as possible. The fact that in these experiments the ants were always sure about the fictive position of the nest (i.e. exhibited sharp search peaks there) shows that contrary to bees in ants the presence of optic flow is not necessary for gauging distances correctly. However, as optic flow whenever present influences distance estimation to a remarkable extent (Ronacher and Wehner, 1995), we designed experiments in which the ants were either provided or not provided with optic flow during their pre-test treatment, but were never provided

with optic flow in the test situation.

4.2.1 Training situation

Desert ants, *Cataglyphis fortis*, were trained to forage to a feeder located 1.5m away from the nest entrance (Fig. 4.1). During the whole time the ants were enclosed in a channel arrangement (profile dimensions: 7 by 7cm) depriving them from external landmark information but presenting them with directional information by skylight cues (width of strip like celestial window: 53°). Animals returning from the feeder with a food item between their mandibles were captured directly at the nest entrance and transferred to one of two different test setups. The training procedure assured that the ants had completely run off their (1.5m) home vector and hence were in their zero-vector state.

4.2.2 Treatment 1

The zero-vector ants were released at the elevated end of an inclined channel (length: 4m; inclination: 52°) oriented orthogonally to the training setup (Figs. 4.1, 4.2). The channel, the interior of which was painted uniformly grey (Experiment A), was made slippery to the ants by covering its interior with a transparent plastic foil. When being released inside the channel, the ants usually directly slid down the inclined 4m channel, and in doing so covered a ground distance of 2.4m (Fig. 4.1: Treatment 1). In order to test, whether they had detected the more or less passive movement, they were picked up at the bottom end of the channel and were released into a horizontal test channel that was parallel to the ground projection of the inclined channel. If the ants were able to monitor the passive displacement and by that integrate it into the ground distance covered (Wohlgemuth et al. 2001), they should have exhibited a nest search not centered on the release point but, ideally, shifted by 2.4m in the direction opposite to the prior experienced displacement (compare Figs. 4.1 and 4.2). We tracked the first eight turning points of each ant's nest search behavior linearly performed within the test channel. In order to be sure that the ants still maintained their homing motivation, ants which while being tested had lost their food crumbs were excluded from further analyses (see Results-section for the success rate of each set).

In another experiment (Experiment B) we increased the optical flow within the inclined channel by providing the interior of the channel with a pattern of black and white stripes (λ=4cm) arranged orthogonally to the sliding movements of the ants. A third group of ants (Experiment C) was released five times consecutively in the inclined and patterned channel before the ants were transferred to the test channel. This procedure enabled us to increase the distance between the fictive position of the nest as defined by the path

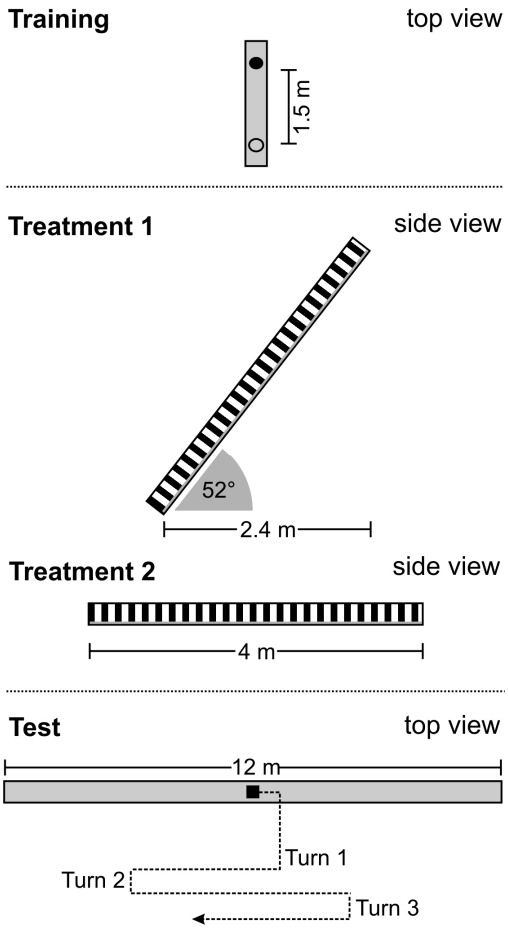

Figure 4.1: Schematic view of the experimental setup. Training took place in a 10m channel with an exit hole on the side, where ants were able to enter and leave the setup and forage to a feeder 9m down the channel. The colored rectangular area denotes the position of the ground landmark during training (PI-position). For tests, the ants were transferred into another channel 18m long and aligned parallel to the training channel. Within this test channel ants were presented with a ground landmark at varying positions (LM-position) relative to the position indicated by their path integrator (PI-position, experiment 1), or with ground landmarks differing from the training landmark in visual or tactile properties (experiment 2). The first six U-turns of the ant's search behavior were recorded. Drawings are not to scale.

integrator if no movement had been detected by the ants (0m) and the fictive position of the nest as defined by the path integrator if movement had been detected (2.4m * 5 = 12m).

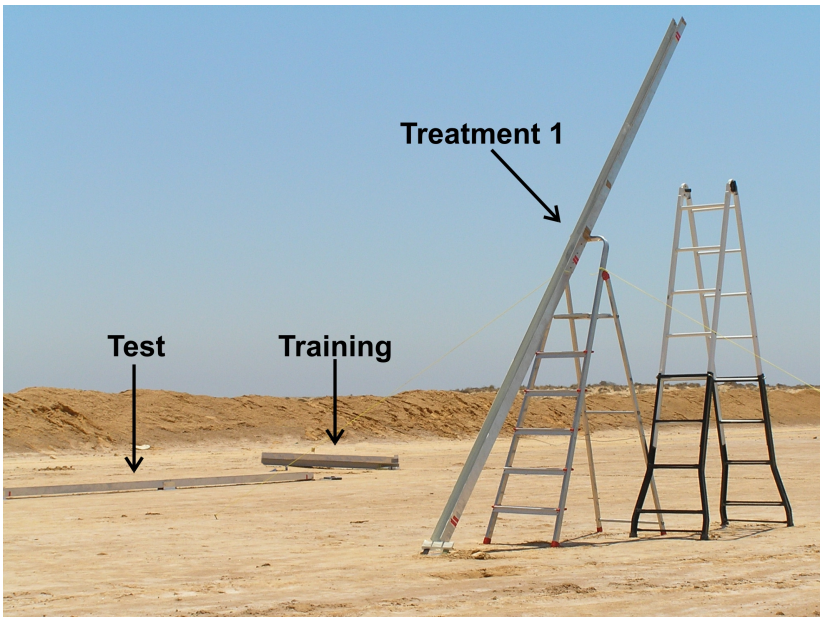

Figure 4.2: Experimental setup including training, treatment 1 and test. Both the test channel and the ground projection of the treatment channel were oriented orthogonally to the training channel.

4.2.3 Treatment 2

In a second set of experiments zero-vector ants were again exposed to passive movements but now by clamping them into the tip of a pipette with their head peering out and the rest of the body (including the legs) being immobilized by the tip (Fig. 4.3). The tip was then fixed at the front of a small vehicle, so that the position of the ant's head relative to the ground corresponded to the normal head position during running (about 44°, Wehner 1982). The vehicle was moved with a speed of about 0.3m/s (corresponding to the ants' normal running speed) within the now horizontally oriented channel (length: 4m, Fig. 4.1: Treatment 2), which was again provided with black and white rectangular grating (Experiment D). By this the immobile ants were exposed to optic flow cues as they would usually perceive them during undisturbed running. In order to increase the

ants' virtual home vector we exposed the ants to five consecutive passive movements in the patterned horizontal channel before we recorded their search patterns (Experiment E). Each ant was tested only once.

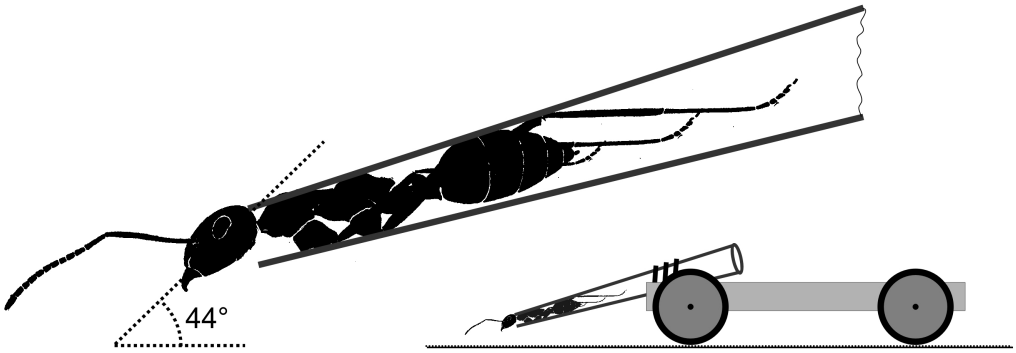

Figure 4.3: Schematic setup of a fixated ant in treatment 2. The ant was fixated in a pipette tip (main figure) and then mounted on a little trolley (insert figure) that was moved through the channel. It was ensured that the ant's head relative to the ground corresponded to the normal head position during running ($\approx 44°$) as observed by Wehner (1982).

4.2.4 Control

As a control, ants were captured at the nest entrance and transferred directly into the test channel. Once tested, they were excluded from further experiments.

4.2.5 Data analysis

We recorded the first eight turns of each ant's nest search. In order to determine the search density distribution of a single animal's search we divided the test channel in virtual bins of 0.1m length and calculated how often each bin had been visited by the ant. We then normalized the obtained data for each ant by dividing the number of visits within each bin by the total number of visits of all bins. The search density distribution of an experimental set shows the mean value of each bin for all ants.

For statistical analysis the center of each animal's search was determined by calculating the median of the visited bins. In all experiments (A-E) we tested whether the search

centers differed from that of the control group by using the Kruskal-Wallis test (α=0.05, Kruskal and Wallis, 1952) accompanied by the Dunn-Sidak Multiple Comparisons post test (Hochberg and Tamhane, 1987). In order to characterise the precision, with which a tested ant located its fictive nest, we determined the search ranges of each animal by following Sommer and Wehner (2005). The values obtained from each experiment (A-E) were tested against the values of the control group using the tests mentioned above.

4.3 Results

The first attempt to disentangle optic flow and active locomotion was to let the ants slide down the inclined and uniform channel. Once an ant was released with a food item it stepped into the channel and almost instantly lost control of its active locomotion. The horizontal component of the 4m displacement was 2.4m. If the ant had integrated its passively encountered movement, the center of its search density distribution should have been located at a distance of 2.4m (dotted line in Fig. 4.4A). However all ants exhibited searches centered on the release point (Experiment A: median=0.1m, n=25 successful runs of 27 trials). This result indicates that the ants had not compensated for their experienced travel (Fig. 4.4A, solid line). It does not differ (Dunn: $p>0.05$) from the control experiment, in which the ants were tested directly after they had been picked up in the training situation (Fig. 4.4A-E: shaded area, median=0.4m; n=25 of 27 trials,).

In Experiment B we introduced a black-and-white stripe pattern underneath the transparent foil, changing the optic impression to the moving ant from a uniform grey to a more obvious pattern. However, although the very same pattern proved to be useful for recognition of self induced optic flow in actively running ants (Ronacher and Wehner 1995), the now prominent optic flow experienced by the sliding ants did not induce the ants to search at a position (Fig. 4.4B: median=0.2m, n=25 of 25 trials) different from the control group (Dunn: $p>0.05$), even if the sliding procedure was repeated five times (Fig. 4.4C: Experiment C, median=-0.15m, n=4 of 5 trials, Dunn: $p>0.05$).

As the ants were often tumbling down the channel rather than sliding in a directed manner, the optic flow on the retina did not always move in a straight direction from

cranial to caudal. In addressing this issue we fixated the ants and transferred each single ant manually through the (in this case horizontally oriented) channel with the animal's head in its natural position. Now the ants experienced an optic flow pattern similar to the one an actively running ant would perceive, but were again refrained from any active locomotion. (Fig. 4.4D: Experiment D, median=0.55m, n=18 of 36 trials). As the search density distribution of the animals did not differ from the one of the control group (Dunn: $p>0.05$), the directed optic flow had not provided the ants' path integrator with any input information. Five repetitions of the procedure mentioned above (Fig. 4.4E: Experiment E, median=-0.55m, $p<0.05$, n=15 of 19 trials) resulted in a significant difference from the search medians of the control group (Dunn: $p<0.05$). However, the difference is small and oriented in the wrong direction i.e. instead of compensating for the 20m displacement, the ants ran in the very same direction in which they had been displaced. In summary, in all experiments, apart from Experiment E, the search centers did not differ from that obtained in the control experiment in which the ants were tested without any displacement treatment.

The median search ranges, i.e. the difference between the extreme points of an ant's nest search behavior, did not differ significantly among the various paradigms from the search ranges of the control ants (median search ranges: control: 2.9m, 1A: 3.0m, 1B: 3.2m, 1C: 3.1m, 1D: 2.3m, 1E: 2.5m; Kruskal-Wallis: p=0.2227). In all experiments the ants located their fictive nest with the same precision, no matter which treatment they had encountered before.

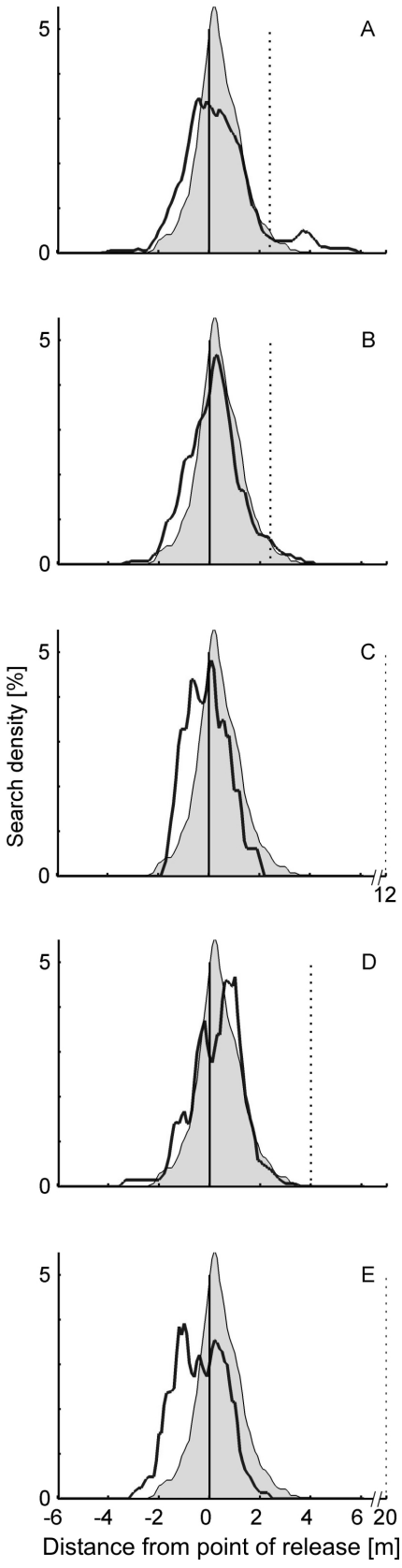

Figure 4.4: Search density distributions of the ants' searches after passive displacement. A. During the experiment the ants were sliding down the slippery and optically homogeneous inclined channel. B. The ants were sliding down the channel as in A, but this time experiencing prominent optic flow by perceiving a contrast-rich black-and-white pattern (rectangular grating). C. The ants experienced the same conditions as in B, but their pre-test treatment was repeated five times. D. Fixed ants experiencing directed optical flow across their eyes while being moved through a horizontal channel. E. The ants were fixed as in D, but driven through the channel five times before being tested. (Shaded area: control experiment of ants that were tested without passive displacement. Solid lines: hypothetical position of the nest entrance if optic-flow information had not been integrated. (0m). Dotted lines: hypothetical position of the nest entrance under the assumption that optic-flow information had been fully integrated.)

4.4 Discussion

Distance estimation is an essential component of path integration (for a review see: Wehner and Srinivasan, 2003). Flying insects use optic flow as their main source for distance estimation (honeybees: Esch and Burns, 1995; stingless bess: Hrncir et al., 2003; wasps: Ugolini, 1987). Using an elegant experimental paradigm Srinivasan et al. (2000) were able to calibrate the bees' odometer. By forcing the bees to fly through a patterned tunnel, the optic flow received by the bees could be quantitatively manipulated, and its influence on the bees' odometer could be quantified by analyzing the distance information played out in the bees' recruitment dances. Depending not only on the distance covered but also on the bees' flying altitude (Esch and Burns, 1995) and on the visual structuring of the environment (Tautz et al., 2004), distance estimation via optic flow is error prone. Contrary to flying bees desert ants walk, and do so within an optically rather homogeneous environment. Hence, as the altitude of the animals above ground and the visual structure of the ants' foraging environment are rather constant, optic flow experienced in the ventral field of view should reveal a good estimation of distance covered. Ronacher and Wehner (1995) tested ants in channels within which ground patterns could be moved either in or against the ants' running direction. Hence the optic flow perceived by the ants in the test situation was either reduced or increased. If ants were exposed to a reduced optic flow during their home-bound runs, they underestimated the distance covered, while they overestimated it, if the optic flow was unnaturally high. However, the effect of pattern movement on the distance estimation was smaller than expected if the ants had relied exclusively on the experimentally manipulated optic flow cues. Furthermore no speed trapping was observed, i.e. the ants did not adjust their running speed to the manipulated optic flow. The fact that ants whose ventral eye hemispheres had been covered with light tight paint (Ronacher and Wehner, 1995) were still able to gauge distances precisely, shows that optic flow is not essential for measuring distances as long as proprioceptive cues exist. In the present account, by preventing the ants from active locomotion, and hence proprioceptive input, but letting them experience optic flow field cues, we set out to test whether optic flow is sufficient for path integration, if proprioceptive cues were eliminated.

Ants are able to calculate the ground distance when they are trained in an array of uphill and downhill channels (Wohlgemuth et al., 2001, 2002). Are they still able to do so if

during translocation they do not move actively, but had to rely on optic flow alone? We released ants within slippery downhill channels and by this forced them to slide down passively instead of walking actively (Fig. 4.4A). Within the channel the ants, when attempting to walk, fell over and then usually slid down the channel in a directed manner with their body not consistently oriented as it would be during controlled locomotion. As the search patterns subsequently performed by the ants in the horizontal test channel did not differ at all from the control ants, the tested ants had not been aware of the experienced displacement, and hence had not included this information into their path integration system. Even if the downhill channel was conspicuously patterned, the ants did not make use of the flow-field information obtained during their passive movements (Fig. 4.4B).

Ugolini (1987) transported wasps, *Polistes gallicus* (L.), in a transparent container to a previously unfamiliar site, allowing them to asses both optic flow and skylight cues. Upon release the wasps headed towards home, seemingly having integrated the passively experienced displacement. As the ants tested in the present account were sliding down the channel, they might not have experienced optic flow field cues coherent enough in directional (cranial - caudal) terms to trigger direction sensitive cells in their visual system (as described, for example, by Egelhaaf and Warzecha, 1999). In order to improve this flow-field situation, we fixated the ants in front of a small vehicle with their eyes in the natural angular position and distance from the ground (Fig. 4.3) and moved them passively through a horizontal channel. By this procedure the immobilized ants experienced naturalistic optic flow cues as freely walking ants would have experienced them and analogous to the optic flow cues recorded and replayed to stationary flies (Kern et al. 2001). Nevertheless, the ants did not take into account the perceived displacement when calculating their position relative to the nest. While bees and wasps might completely rely on optic flow information for path integration, ants have been shown to do so only to a minor extent. However, even when this minor effect had been increased by repeating the pre-test sliding treatments five times (Experiments C and E), the ants continued to search for the nest as if they had not integrated any optic flow field cue at all.

If distance estimation via optic flow and via proprioceptive cues were part of a redundant odometric system, one would expect that the ants were able to detect their passive displacement visually and hence move the centers of their searches either 2.4m (Exper-

iments A, B), or 4m (Experiment D), or even further away from their starting point (Experiment C: 12m, Experiment E: 20m). Such was not the case. The ants searched at the very same place as the non-displaced control ants did, and hence completely ignored the displacement which they could have deduced from the visual movement of the surroundings experienced during their passive displacements. Obviously, *Cataglyphis* ants use optic flow field cues for distance estimation (as shown by Ronacher and Wehner 1995) only if they move actively and hence simultaneously gain information from their motor control system.

Might the missing influence of the optic flow just be due to the ants' confusion caused by the experimental treatments? In this case we would not have expected the ants to directly pick up a food item (i.e. to be still in their foraging mood). Furthermore we would have expected search patterns that differed in their spatial spread from that of control ants. As this was not at all the case, the missing integration of optic flow, rather than merely a state of confusion by the treatments seems to have been the major component responsible for our results.

In polydomus ants experienced foragers can be observed carrying indoor workers from the principal nest housing the queen to newly founded satellite nests. When the carrier ant and the carried ant get separated, and when both ants are transferred to an unfamiliar test area, the carrier continues running in the new direction, while the carried ant walks back in the direction of the original nest. Obviously, the latter ant has obtained directional information while being carried (*Cataglyphis bicolor*: Duelli, 1976; *Cataglyphis iberica*: Fourcassie et al., 2000). The carried ants even had some coarse knowledge of the distance over which they had been carried (Duelli, 1976). They might have acquired this knowledge by measuring the time they had been carried, but as carrier and carried ants adjust their head inclination, and hence the tilt angle of their eyes, in exactly the same way, processing of optic flow might also have played a role in the absence of proprioceptive input. As in the current experiments proprioceptive cues were eliminated as well, this time experimentally, an influence of passively experienced optic flow-field cues could have been expected on the basis of the results obtained in ants that had been carried by nest mates. However, not the least bit of such an influence had been observed. In conclusion, whatever the contribution of visual flow-field information to the ant's odometer might be, such a contribution is present only if the ants are walking actively rather than being displaced passively.

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5 How do desert ants integrate inclination?

Abstract During long-distance foraging in almost featureless habitats desert ants of the genus *Cataglyphis* employ path-integrating mechanisms (vector navigation). This navigational strategy requires an egocentric monitoring of the foraging path by incrementally integrating direction, distance, and inclination of the path. Monitoring the latter two parameters involves idiothetic cues and hence is tightly coupled with the ant's locomotor behavior. In a kinematic study of desert ant locomotion performed on differently inclined surfaces we aimed at pinpointing the relevant mechanisms of estimating step length and inclination. In a behavioral experiment with ants foraging on slippery surfaces we broke the otherwise tightly coupled relationship between speed and step length and examined the animals's ability to monitor distances covered even under those adverse conditions. The results of the experiments show that the ant's locomotor system is not influenced by inclined paths and stepping parameters such as step length, and stepping frequency are solely dependent on speed of locomotion. In consequence the previously proposed monitoring of joint angles does not serve for inclinometry. We also argue that an efference copy of the central pattern generator coding the step length in its output frequency will also not suffice for estimating step length and complement the pedometer. In conclusion we propose that sensing forces acting on the ant's legs could provide the desired neuronal correlate employed in monitoring inclination and step length.

Key words path integration, slope detection, odometer, locomotion, *Cataglyphis fortis*, *Formica pratensis*

5.1 Introduction

Foraging desert ants, *Cataglyphis fortis* Forel 1902 (Wehner, 1983), can navigate without using local external cues such as odor trails or landmarks. Instead they employ mechanisms of path integration (Mittelstaedt and Mittelstaedt, 1982), also called vector navigation (Wehner, 1982), which requires that the ant constantly updates its home vector, i.e. the location of the colony's nest defined by the direction and distance with respect to the animal's current position. In order to gather this information the ant must integrate each single increment of the path covered into its navigational system (Mittelstaedt and Mittelstaedt, 1980; Wehner and Srinivasan, 1981). Therefore it must continually monitor the directions steered and distances covered in these directions. While compass information is gained by exploiting celestial cues (e.g. Wehner and Lafranconi, 1981; Wehner, 1989; Wehner, 1994b; for a review see: Wehner and Srinivasan, 2003; Wehner and Labhart, 2006), the mechanism of distance estimation has been a long standing problem. Flying insects rely on visual flow field cues (bees: Esch and Burns, 1995; wasps: Ugolini, 1987), but desert ants completely disregard lateral visual cues (Müller and Wehner, unpublished data) and incorporate ventral ones only to a degree of about 10 per cent (Ronacher et al., 2000; Ronacher and Wehner, 1995). Energy expenditure as a measure of distance, as proposed by Heran and Wanke (1952), can be ruled out as well, because in ants loaded with food items on their inbound paths distance measurement is not impaired (Schäfer and Wehner, 1983). With an elegant experiment Wittlinger et al. (2006) proved a hypothesis first proposed by Pieron (1904) that some kind of step counter is responsible for distance estimation. However, as step length varies with speed of locomotion (Zollikofer, 1994a), a mere step counter will not work (Wittlinger et al., 2007a). In order to calculate a distance correctly, both step number and corresponding step length need to be integrated. So far it is unclear how step length is monitored in desert ants. According to Zollikofer (1988) and Wittlinger (2007a), step length and step frequency are tightly coupled within an individual ant. Hence, the output frequency of the central pattern generator (CPG) may already contain the information required for distance estimation.

But vector navigation does not only take place on level ground: the ant's path integration system is able to compensate for vertically corrugated paths. Wohlgemuth et al. (2001, 2002) showed that ants were able to correctly calculate the ground distance between

feeder and nest even if the ants had performed their previous outbound runs on a corrugated surface and hence had covered much longer paths. The animals had to have a representation of the slope of their outbound path and based on this representation had computed the corresponding ground projection (base line) of the three-dimensional path. The mechanisms responsible for slope detection most likely involve idiothetic cues. For obvious reasons, energy expenditure will be a misleading cue as running up and down a corrugated path will require more energy than to take the short and level projection path. Visual input by skylight cues which change their position in the visual fields of uphill and downhill running ants were also excluded by Wohlgemuth et al. (2002). In insects, slope (via gravity) can be monitored by idiothetic cues either by hair field sensors between body parts (Markl, 1962) or by campaniform sensilla within the cuticle of the legs of running insects (Horn, 1985). As Wittlinger et al. (2007b) had deactivated hair sensilla on the ant's body without causing any impairment on the ants' ability to integrate their paths on corrugated sheets, we now focus on the legs and their movement parameters as a source of information potentially used by the ants, i.e. in measuring the inclination of the surface on which they walk.

In summary, idiothetic cues are the most plausible mechanisms for measuring both step length and slope in three-dimensional path integration of desert ants. In general, we consider three types of parameters and their possible use in three-dimensional path integration: (i) Does the angular working area of the legs vary with inclination, including a resulting change in step length? (ii) Do temporal patterns such as swing period and stance period depend on the inclination of the walking floor? Finally, (iii) do forces acting along the legs during the stance phase of the stepping cycle change with inclines and with step length, and hence would be able to serve as a cue for three-dimensional path integration? We designed two experiments addressing these questions: First, we studied the kinematics of leg movements of ants running on differently inclined surfaces. This study should show whether the first two groups of parameters mentioned above, i.e. geometric and temporal variations of leg movements, played a role. Second, we performed a behavioral experiment with ants foraging on slippery substrates and therefore being prevented from producing high mechanical forces along their legs. This study should show whether force-monitoring is a mechanism employed in path integration.

5.2 Materials and Methods

5.2.1 Kinematic analysis

High-speed video recordings were performed in August 2004 in our field station in Maharès in southern Tunisia (nest coordinates: N 34° 31.745', E 10° 32.333') in desert ants, *Cataglyphis fortis*, and in October 2003 in Jena (Germany) on wood ants, *Formica pratensis* (Black-Backed Meadow Ant, Retzius 1783). We used active foragers of established colonies, which were linked to a channel array. The animals were trained to forage to a feeder at the end of a U-shaped aluminum channel (profile dimensions: 7 by 7cm). During foraging, the ants were recorded on their outbound paths when they passed a narrowed part of the channel. In addition, the inclination of this part of the channel could be varied, so that the ants foraging on differently inclined floors could be recorded (Fig. 5.1).

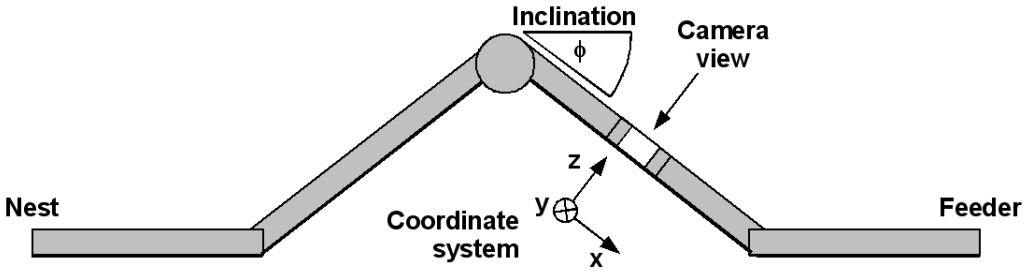

Figure 5.1: Schematic drawing of the setup of experiment 1 (side view). While foraging from the nest to the feeder the ants passed a narrowed section of the channel, within which they were recorded from above (x-y-plane, see arrow). The inclination of this section could be altered from level ground (ϕ=0°) to almost vertical (ϕ=90°). In a complementary study, lateral views (x-z-plane) were recorded and are analyzed by Tom Weihmann.

Data acquisition and analysis

The ants' movements were recorded with a high speed camera (Redlake PCI 2000 S) at 250 frames per second. Approximately 100-150 sequences of freely foraging outbound ants were taken for each inclination. The nests used for this experiment contained about 70 to 100 active foragers per day. For kinematic analysis only sequences with straight

and uninterrupted runs were chosen. The sequences were tracked in the laboratory of Reinhard Blickhan (Friedrich Schiller University Jena, Germany) using WinAnalyze (Mikromak) tracking software. For the analysis of the stepping parameters the head-thorax joint and the petiolus were tracked as well as the contact point of the tarsi of all six legs with the ground (Fig. 5.2). Prior to each series of recordings the setup was calibrated by recording small plastic blocks (Lego systems) that filled almost the entire field of view. During tracking, data were calibrated by a linear measure of the calibration body. Raw data were exported and analyzed using custom written programs implemented in Matlab (The Math Works Inc.). The center of mass (COM) of each animal was defined as 1/10 of the vector from the petiolus to the head-thorax joint. This caused the COM to rest between the coxae of the middle and the hind leg pair (see also Zollikofer, 1988). The origin of the coordinate system was transferred to the COM with the x-axis being aligned to the body axis. Additionally, for each sequence analyzed the width of the ant's head was determined from the video sequences. This measure is linearly correlated with body size (Wehner, 1983). We refrained from calculating values for a standardized body model as (i) the scaling factors differ slightly between the front, middle, and hind legs and also between the two species. Furthermore (ii) as we took freely foraging animals, we were not able to take measurements on, e.g., alitrunk length or leg length. However, in dorsal recordings the absolute head width can be easily determined and can serve in our model as a covariant for individual body size.

Modeling stepping parameters

Stepping patterns are influenced by speed, but my also vary between differently sized animals and between animals of different species. In order to separate these effects from a possible effect that slope has on stepping patterns, we analyzed each examined parameter (see Tabs. 5.1, 5.2, first column) with the four input variables speed, head width, species, and slope. As the data set is unbalanced, we performed fits with reduced maximum likelihood-methods (LME-function, NLME-package; Pinheiro and Bates, 2000) implemented in the statistical software package R (`www.r-project.org`) in the following form:

$$Parameter = \alpha * Speed + \beta * Species + \gamma * Headwidth + \delta * Inclination + \epsilon$$

The input-variable 'Species' is a factor and not a value and hence the animals are ordered alphabetically. A negative β indicates that the fitted parameter is higher in *C. fortis* than in *F. pratensis*. All other variables used throughout this study are numeric. Goodness of fits can be compared with the log-likelihood given for each fit (Tabs. 5.1, 5.2, last column), with a value indicating a better fit (assumed identical degrees of freedom, which is the case in our analyses). Steps within one single run were nested, i.e. not treated independently. Each single input variable fitted to a certain parameter has its own level of significance. In the results we only mention fitted parameters with $p<0.1$. For most of the parameters, data analysis was focussed on front, middle, and hind leg irrespective of the left or right side of the body. Footfall geometry was analyzed on legs that belonged to one tripod of the typical gait of fast running insects (and also *Cataglyhis* ants, see

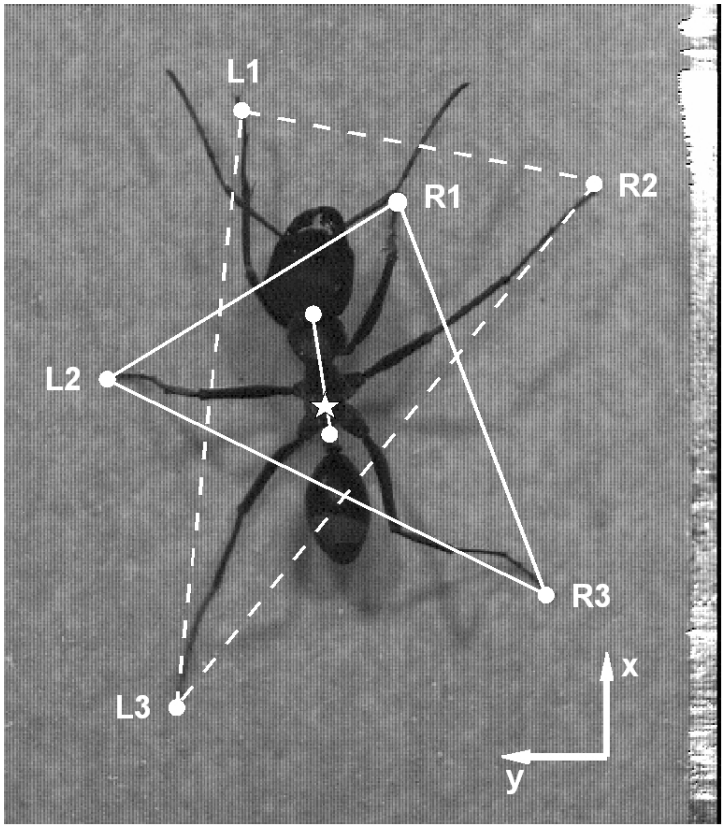

Figure 5.2: Dorsal view of *C. fortis* foraging in the channel arrangement. For kinematic analysis the head-thorax joint and the petiolus were tracked as well as the contact point of the tarsi with the ground of all six legs (L1 through R3 for left and right, front to hind leg respectively).

Zollikofer, 1988). Hence we chose the right front leg (R1), the left middle leg (L2), and the right hind leg (R3), which together form one of the ant's locomotive triangles. For the analysis of phase relationships, we determined the phase shift of all legs with respect to the left middle leg (L2).

5.2.2 Behavioral Experiment

Cataglyphis fortis ants were trained to forage to a feeder 9m from the nest entrance. During the whole foraging excursion the animals were enclosed in a linear flat channel (material and dimensions: see above), in which the ground was covered with very fine abrasive paper (grit size: 100nm; Microtec, Sia Abrasives) that minimized frictional forces between tarsi and ground (Dai et al., 2002). This analogue to a larger animal walking on ice causes the ants to walk with highly increased slip rates, especially during phases of acceleration and deceleration. In order to amplify the slip rate even further, the foraging runs took place under different wind conditions (head wind and back wind; both 3-4m/s; at wind speeds above 5m/s *Cataglyphis* will usually not initiate foraging runs, personal observations) resulting in an extreme increase or decrease of the number of steps required by the animal in order to cover the same 9m nest-feeder distance. The interior of the channel (walls: spray-painted with grey varnish, ground: 100-nm grit abrasive paper) did not provide the ant with any visual flow field cues that could have provided a feedback on running efficiency. When an ant had reached the feeder and had taken a food item (usually melon flavored biscuit crumbs) it was transferred to a parallel test channel that was twice as long as the training channel and contained a rough ground. Once released, each ant directly ran off its home vector, i.e. the representation of the distance from the feeder to the nest entrance. When the home vector had reached zero-state, the ant started a symmetric search centered on the expected nest entrance. Six turns within the linear search pattern of each ant were recorded, and search density distributions were computed for each experimental set (for details see Seidl and Wehner, 2006).

5.3 Results

5.3.1 Locomotion on inclines

We recorded 263 sequences, each of which contained one to twelve steps (mean: 3 steps) analyzed resulting in a total of 876 steps. Separated by species and inclination this amounts to: -60°: 47 and 67 steps (*C. fortis* and *F. pratensis*, respectively), -30°: 33 and 181 steps, 0°: 54 and 127 steps, 30°: 54 and 186 steps, 60°: 64 and 63 steps. Negative inclinations denote downhill paths. The examined ants ran at speeds between 0.05 and 0.4m/s. However, the effect of slope on speed of locomotion differs between the two species (Fig. 5.3). While desert ants reached maximum speeds on level ground (0.4m/s or about 40 body lengths per second), the wood ant accelerated most on modest downhill paths (0.25m/s, -30°). On steep uphill paths (60°) both ants ran at the same speed of 0.1m/s, but differed extremely on steep downhill slopes (-60°): 0.07m/s (wood ant) and 0.23m/s (desert ant).

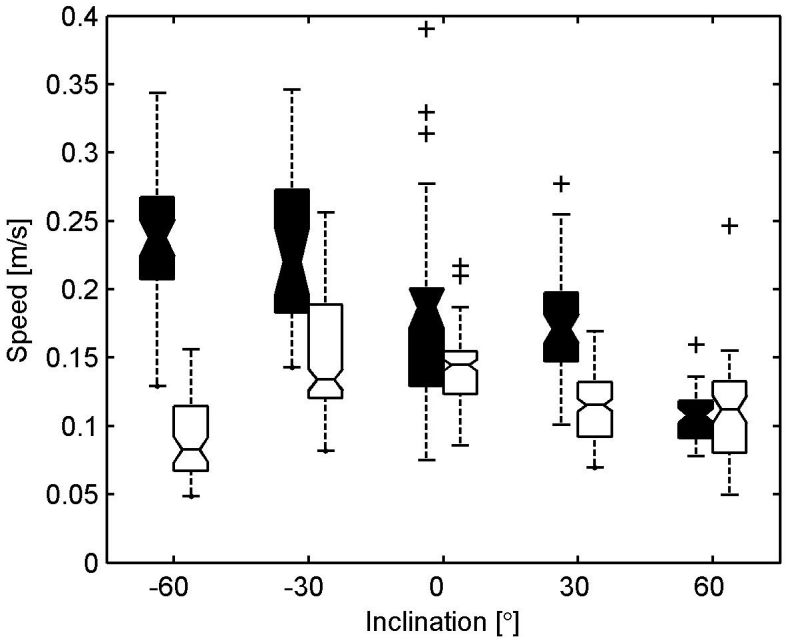

Figure 5.3: Speed of locomotion of *C. fortis* (filled boxes) and *F. pratensis* (empty boxes) at different inclinations. (Boxplots with 25%, 50% and 75% percentiles; whiskers extend to max. 1.5 times the inter-quartile range.)

The major aim of this study is to identify effects of the inclination of the walking floor on the kinematics of the ant's locomotor behavior. These effects could help to identify possible mechanisms for three-dimensional path integration. In order to differentiate between the sources of observed effects each kinematic variable was modeled with four input variables: speed of locomotion, species (considering allometric morphological variation between species), head width (considering within-species size effects) and slope. The model parameters including the corresponding p-values are listed in (Tab. 5.1, 5.2). The results presented in the following are grouped according to six different hypotheses on path integration on inclines. As mentioned above, ants are able to compute ground distance covered on a hilly path, and hence must be able to monitor the inclination of the floor on which they walk.

Geometric parameters

Our first hypothesis on the mechanisms of path integration on inclined paths states: "*With increasing slope, path-integrating ants increase their step length in such a way that for a given base-line distance covered the number of steps remains constant irrespective of the inclination of the floor on which the ants have actually walked.*" Hence, in a first analysis we modeled step length with absolute inclination, i.e. did not differentiate between uphill and downhill runs. The desert ants made steps of 10.1±2.5mm (all values: mean ± s.d.), while the wood ants had step lengths of 7.9±1.3mm (Fig. 5.4). Desert ants make longer steps than wood ants make, hence the species effect has a negative value: $\beta \approx$–0.4mm (compare Material and Methods section, page 66; Tab. 5.1). In addition there is also an effect of individual body size: larger individuals made longer steps ($\gamma \approx$0.8mm step length per 1mm head width), i.e. an animal with a head 1mm wider has steps that are 0.8mm longer than those of a comparable ant. The major effect on step length, however, is speed of locomotion ($\alpha \approx$29mm per m/s), i.e. an ant accelerating for 1m/s elongates its steps for 29mm. Slope, however, has a significant influence on step length only in the hind leg ($\delta =$–0.0063±0.003mm per degree inclination). An increase of inclination of e.g. 30° would result in a shortening of the step of 0.2mm, or 1.9% in *Cataglyphis fortis*. (Note: The model parameter is significant in statistical terms. The value of the parameter however is - in this specific case - small and hence insignificant in biological terms.) The influence of inclination on step length is also visualized in Figure

5.4: The inclination wise linear fits of step length vs. speed do not differ considerably. Our first hypothesis - steps get longer with absolute inclination - is herewith rejected. Even if step length is modeled with the distinction between uphill and downhill runs, as done in all following analyses, the δ-values do not become significant; all other model-parameters remain fairly the same.

Walking on an inclined surface requires more force, because lift of the whole body has to be generated. While running on level ground, the vector of gravity is oriented vertically to the surface and hence is normal to the horizontally produced thrust. On inclines however, there is always a component of the gravitational force acting along the direction of locomotion, pulling the ant forward or backward (see also Fig. 2.3). Hence, there is additional acceleration acting on the body which has to be integrated while generating the desired forward thrust. In order to maintain static stability, desert ants could adapt their locomotive behavior by moving the stance phase in direction of the gravity vector, similar to a person tilting its body towards a strong wind. These considerations lead to our second hypothesis on path integration in the third dimension: "*On uphill paths the footfall positions are moved backwards (caudally) and on downhill paths they are moved forwards (cranially).*" Such a behavior would allow the ant to keep its COM longer in the static safe position above the supporting tripod-triangle. After a swing phase, the tarsi reach their most forward point just when they touch down. At this phase of the stepping cycle, the anterior extreme position (AEP), most of the variation that can be observed is due to the species-effect: In relation to desert ants, wood ants position their front legs backwards (β=–0.74$\pm$0.16mm) and inwards (β=–1.18$\pm$0.12mm), their middle legs are placed slightly forward (β=0.38$\pm$0.18mm) and inwards (β=–2.02$\pm$0.13mm), while their hind legs touch the ground both in a forward (β=1.78$\pm$0.17mm) and inward position (β=–1.19$\pm$0.12mm). These species effects can be observed with similar values at the end of the stance phase, the posterior extreme position (PEP): Again in relation to the desert ant, the wood ants' front legs move backward and inwards (β=–0.81$\pm$0.11 and -1.4$\pm$0.11mm), the middle leg slightly forward and inwards (β=0.63$\pm$0.13 and -1.68$\pm$0.13mm), and the hind leg forward and inwards (β=2.01$\pm$0.16 and -1.45$\pm$0.13mm). The individual body size of an ant within one species has significant (or in tendency) influence in four cases: The front leg is moved forward both in AEP and PEP (γ=0.58$\pm$0.25 and 0.46$\pm$0.17mm per mm head width). The middle leg is, in tendency, positioned forward (γ=0.47$\pm$0.27mm

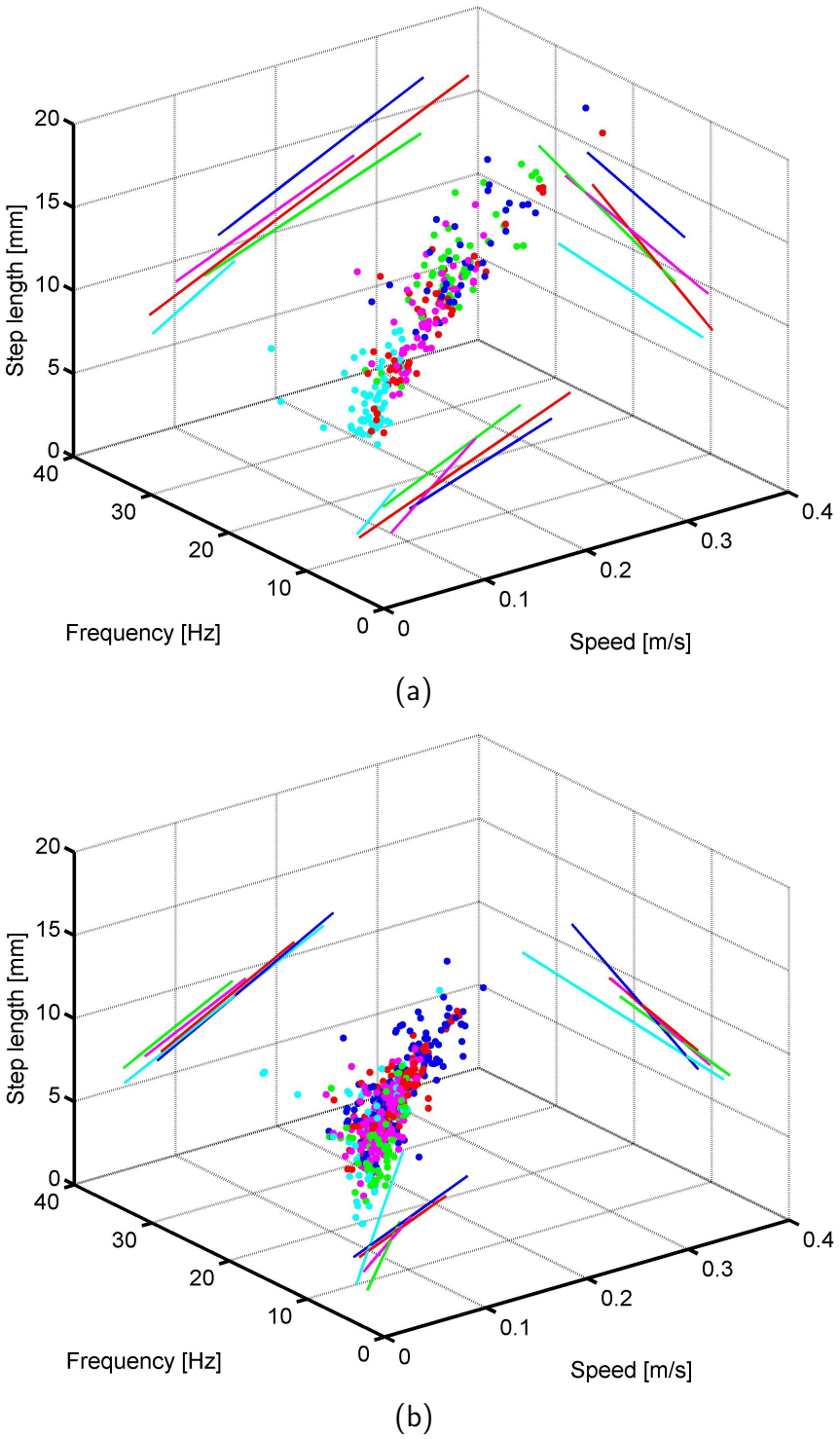

Figure 5.4: Relationship between speed, step length and stepping frequency at different inclinations in *C.fortis* (a) and *F. pratensis* (b). (Dots: values plotted in 3D space; Lines: projected linear fits of data on two parameters; green: -60°, blue: -30°; red: 0°; magenta: 30°, cyan: 60°).

per mm head width) in AEP; the hind leg is positioned backward (γ=–0.59±0.25mm per mm head width) in PEP. Fast running ants place their front and middle leg forward (α=3.99±1.07 and 3.13±1.2mm per m/s) as well as the middle leg outward during touch down (α=3.02±0.83mm per m/s). At the end of the stance phase (PEP), high speed of locomotion induce ants to move their middle legs further backwards (α=–1.83±0.84mm per m/s), as well as their front legs inwards (α=–1.44±0.74mm per m/s) and their middle leg outwards (α=4.09±0.82mm per m/s). Slope, however, has a significant effect on AEP and PEP geometry only on the front legs: in both cases the front tarsus is positioned inwards (δ=–0.002±0.001 and –0.003±0.001mm per degree inclination). A graphical display of the data is shown in Figure 5.5; the different effects on the positioning of the tarsi during AEP and PEP are shown in Fig. 5.6. There is no visible forward or backward shift of AEP or PEP which could indicate an inclination-dependent response. Hence, our second hypothesis can clearly be rejected. Geometric factors such as step length or the standing triangle of the tripod do not vary with inclines. In other words, monitoring the positions of the legs does not provide the ants with information on the inclination of the substrate on which the animal is walking.

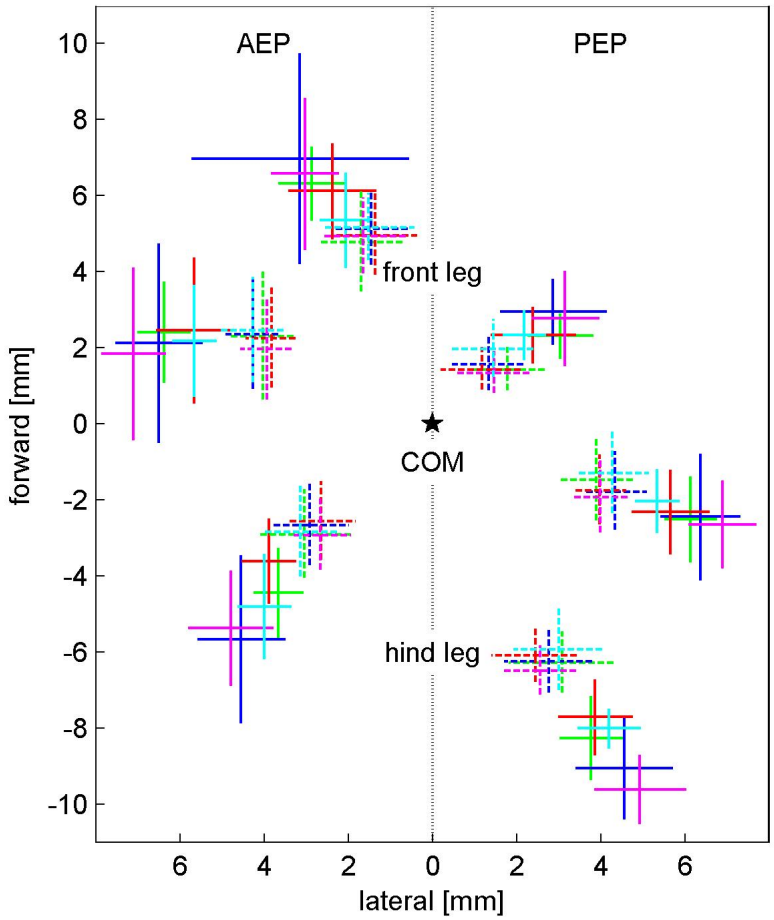

Figure 5.5: Footfall geometry of *C. fortis* (solid lines) and *F. pratensis* (dashed lines) during touch down (AEP) and lift off (PEP) at different inclinations. The intersection of two lines denotes the mean, while the ends of the lines denote the standard deviation. (Star: center of mass (COM); green: -60°, blue: -30°; red: 0°; magenta: 30°, cyan: 60°)

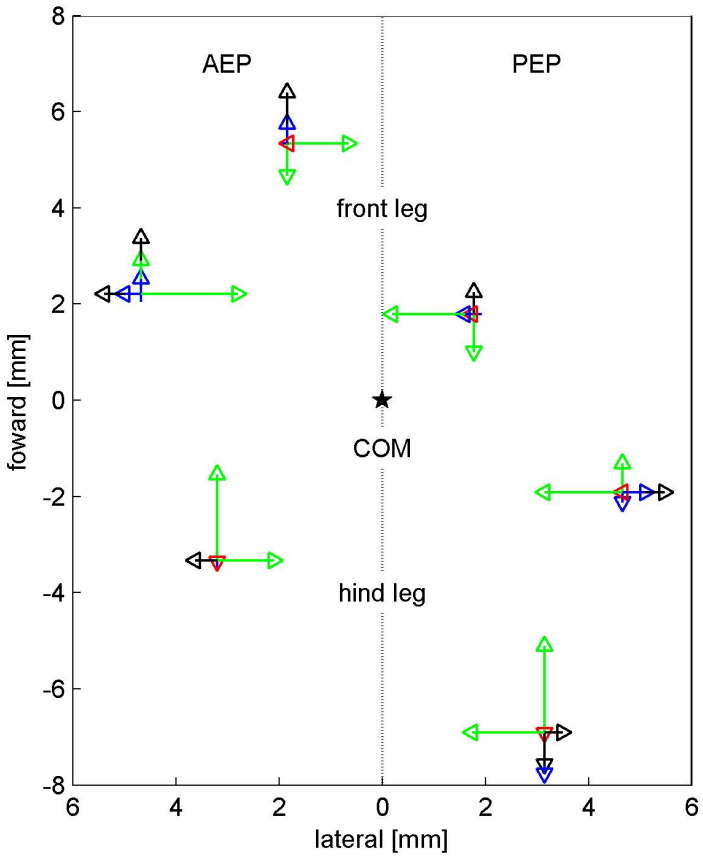

Figure 5.6: Influence of the input parameters on footfall geometry during touch down (AEP) and lift off (PEP) phase at different inclinations. The influence of speed (blue, per 0.1m/s acceleration), species (green, from *C. fortis* to *F. pratensis*), head width (black, per 1mm increase in head width), and inclination (red, per an increase of 10° in inclination) are plotted on the mean value of all runs analyzed. (Arrows are attached to each other, i.e. if pointing in the same direction, one arrow initiates at the tip of the previous one.)

Table 5.1: Influence of the four input parameters on geometric kinematic parameters. (Input parameters: 258 degrees of freedom, intercept: 613 degrees of freedom)

Parameter	Leg	α Speed [m/s]		β Species [Cf,Fp]		γ Headwidth [mm]		δ Inclination [°]		ϵ Intercept		log-likelihood
		mean±s.d.	p	mean±s.d.	p	mean±s.d.	p	mean±s.d.	p	mean±s.d.	p	
Step length[2] [mm]	front	29.8±1.4	****[1]	-0.51±0.22	*	0.55±0.34	-	-0.005±0.003[2]	-	3.96±0.77	****	-1322
	middle	28.7±1.2	****	-0.50±0.20	*	0.85±0.30	**	-0.004±0.003[2]	-	3.53±0.68	****	-1467
	hind	29.7±1.2	****	-0.33±0.20	t.	0.72±0.30	*	-0.006±0.003[2]	*	3.55±0.69	****	-1424
Step length [mm]	front	30.1±1.4	****	-0.46±0.22	*	0.57±0.34	t.	-0.0005±0.0019	-	3.67±0.76	****	-1324
	middle	29±1.3	****	-0.45±0.2	*	0.87±0.3	**	-0.0002±0.0017	-	3.29±0.68	****	-1469
	hind	30.1±1.3	****	-0.27±0.2	-	0.74±0.31	*	-0.0007±0.0017	-	3.21±0.69	****	-1427
Cranial AEP [mm]	front	3.99±1.07	***	-0.74±0.16	****	0.58±0.25	*	0±0.0014	-	4.23±0.56	****	-1459
	middle	3.13±1.2	**	0.38±0.18	*	0.47±0.27	t.	-0.0002±0.0015	-	0.65±0.61	-	-1653
	hind	0.1±1.09	-	1.78±0.17	****	-0.33±0.25	-	-0.0019±0.0014	-	-4.05±0.57	****	-1446
Lateral AEP [mm]	front	-0.6±0.81	-	-1.18±0.12	****	-0.07±0.18	-	-0.0022±0.001	*	2.9±0.41	****	-1309
	middle	3.02±0.83	***	-2.02±0.13	****	0.16±0.2	-	0.0001±0.0011	-	5.39±0.45	****	-902
	hind	0.86±0.8	-	-1.19±0.12	****	0.3±0.19	-	0.0011±0.0011	-	3.39±0.42	****	-1115
Cranial PEP [mm]	front	0.68±0.71	-	-0.81±0.11	****	0.46±0.17	**	0.0014±0.0009	-	1.42±0.37	****	-950
	middle	-1.83±0.84	*	0.63±0.13	****	0.2±0.19	-	0.0001±0.0011	-	-2.47±0.43	****	-1311
	hind	-0.4±1.03	-	2.01±0.16	****	-0.59±0.25	*	-0.0008±0.0014	-	-7.24±0.56	****	-1104
Lateral PEP [mm]	front	-1.44±0.74	t.	-1.4±0.11	****	-0.06±0.17	-	-0.0031±0.001	**	3.1±0.38	****	-1182
	middle	4.09±0.82	****	-1.68±0.13	****	0.23±0.2	-	0.0009±0.0011	-	4.86±0.44	****	-961
	hind	-0.29±0.86	-	-1.45±0.13	****	0.28±0.2	-	0.0005±0.0011	-	3.71±0.45	****	-1289

[1]) Levels of significance: **** p<0.0001; *** p<0.001; ** p<0.01; * p<0.05; t. p<0.1; - p≥0.1. [2]) Absolute inclination.

Temporal parameters

Legged locomotion is performed by a cyclic backward and forward movement of the animals' legs. During the backward movement of the legs, i.e. the stance phase, thrust is generated and the animal is pushed forward. During the swing phase the legs are lifted up into the air and brought forward in order to initiate a new stance phase. During this swing phase, the thin and light legs are only lifted against gravity and then swung horizontally. If the animal runs on an inclined path, gravity acts on the animal's body pulling it towards the surface of locomotion as well as downhill along the surface. The swinging legs will have to work against (or with) an additional acceleration pulling the legs backward or forward. This situation does not occur during level running. The mechanically otherwise uncoupled leg could function as a detector for external accelerations, similar to the widely spread antennae on the ant's head or even to the acceleration sensitive and actively moved halteres of dipteran and strepsipteran insects. Hence our third hypothesis on monitoring gravity for path integration reads: "*On uphill paths, swing periods will be longer than on level ground, as additional lift needs to be generated. Accordingly, swing periods will be shorter on downhill paths.*"

Swing periods in desert ants amount to 0.027±0.004s and in wood ants to 0.028±0.006s. The swing periods of the front legs differ between the two species significantly (β=–0.0024±0.0011s for wood ants). The swing period is significantly reduced by speed of locomotion (α≈–0.048s per m/s, Tab. 5.2), and is reduced in tendency by body size (γ≈–0.0032s per mm head width). Additionally there is a significant influence of inclination on swing period (δ≈–0.00002s per degree inclination). If in *Cataglyphis fortis* the slope is increased by, e.g., 30°, the swing period is shortened by 0.0006s or 2.2%. Contrary to our hypothesis, swing period is reduced on uphill paths and elongated on downhill paths. Above all, however, the influence of slope on swing period is very small, so that the effect of gravity on the ant's swinging leg can be neglected. A graphical representation of these findings is shown in Figure 5.4: The inclination wise linear fits of stepping frequency versus speed of locomotion do not differ in either intercept or slope.

Table 5.2: Influence of the four input parameters on temporal kinematic parameters. (Input parameters: 258 degrees of freedom, intercept: 613 degrees of freedom)

Parameter	Leg	α Speed [m/s]		β Species [Cf,Fp]		γ Headwidth [mm]		δ Inclination [°]		ϵ Intercept		log-likelihood
		mean±s.d.	p	mean±s.d.	p	mean±s.d.	p	mean±s.d.	p	mean±s.d.	p	
Cycle period [s]	front	-0.2±0.01	****[1]	-0.004±0.002	t.	0.002±0.003	-	-0.00005±0.00002	**	0.09±0.01	****	2638
	middle	-0.22±0.01	****	-0.005±0.002	*	0.003±0.003	-	-0.00007±0.00002	***	0.92±0.01	****	2585
	hind	-0.23±0.01	****	-0.004±0.002	t.	0.003±0.003	-	-0.00006±0.00002	***	0.09±0.01	****	2569
Frequency [Hz][2]	front	54.9±4.6	****	0.9±0.71	-	-0.29±1.07	-	0.013±0.006	*	9.3±2.4	***	-2590
	middle	58±2.9	****	0.4±0.43	-	-1.21±0.65	t.	0.006±0.004	-	10.8±1.5	****	-2365
	hind	56.9±3.4	****	0.16±0.52	-	-1.22±0.78	-	0.006±0.004	-	11±1.8	****	-2446
Swing period [s]	front	-0.051±0.007	****	-0.0024±0.0011	*	0.0027±0.0016	t.	-0.00002±0.00001	*	0.033±0.004	****	3162
	middle	-0.043±0.007	****	-0.0003±0.0011	-	0.0038±0.0016	*	-0.00003±0.00001	**	0.024±0.004	****	3235
	hind	-0.051±0.008	****	-0.0012±0.0012	-	0.0033±0.0018	t.	-0.00002±0.00001	t.	0.032±0.004	****	3015
Stance period [s]	front	-0.15±0.01	****	-0.0009±0.0015	-	-0.0003±0.0022	-	-0.00004±0.00001	**	0.056±0.005	****	2691
	middle	-0.18±0.01	****	-0.004±0.0016	*	0.0001±0.0024	-	-0.00003±0.00001	*	0.067±0.005	****	2607
	hind	-0.18±0.01	****	-0.0028±0.0017	t.	0.0002±0.0026	-	-0.00004±0.00001	**	0.061±0.006	****	2683
Duty Factor [-]	front	-0.73±0.09	****	0.03±0.013	*	-0.015±0.02	-	-0.00008±0.00011	-	0.63±0.05	****	684
	middle	-0.75±0.1	****	-0.012±0.015	-	-0.035±0.022	-	0.00006±0.00013	-	0.77±0.05	****	672
	hind	-0.95±0.1	****	0.003±0.015	-	-0.012±0.023	-	-0.00012±0.00013	-	0.68±0.05	****	658
Within tripod phase shift [-]	R1	3.78±0.66	****	0.39±0.1	***	0.16±0.15	-	0.0011±0.0009	-	1.9±0.3	****	-1093
	R3	-0.81±0.66	-	-0.07±0.1	-	-0.03±0.15	-	0.0001±0.0009	-	3.5±0.3	****	-1072
Alternate tripod phase shift [-]	L1	1.98±0.45	****	0.18±0.07	**	0.05±0.1	-	-0.0001±0.0006	-	-0.4±0.2	t.	-686
	R2	0.22±0.62	-	0.08±0.09	-	0.05±0.14	-	0.0005±0.0008	-	2.7±0.3	****	-1084
	L3	-0.96±0.42	*	-0.22±0.06	***	-0.11±0.1	-	-0.0007±0.0005	-	0.8±0.2	***	-712

[1]) Levels of significance: **** $p<0.0001$; *** $p<0.001$; ** $p<0.01$; * $p<0.05$; t. $p<0.1$; - $p\geq0.1$. [2]) Frequency is the inverse of cycle period which was actually the directly analyzed value.

However, what happens to the animal's entire body, which obviously weighs a manifold (≈20 times) of a single leg? In this case, much more energy is required to produce lift, i.e. potential energy. On the other hand this potential energy can be released on downhill paths and enables the animal to make use of this external source of energy. Based on this consideration, our fourth hypothesis reads: "*On uphill paths the stance period will require more time as additional lift for the entire body needs to be generated. To the contrary, a shortening of the stance period is to be expected on downhill paths.*"

In *Cataglyphis* ants, stance periods take 0.032±0.011s, while in *Formica* ants the duration of the stance period is 0.039±0.012s. The major influence on stance period is speed of locomotion (α≈–0.017s per m/s), but there is no significant effect of body size. Between species, wood ants display a shorter stance period in the middle leg (β=–0.004±0.002s). Inclination has a significant effect on the stance period in all three legs (δ≈–0.00004s per degree inclination). Slope difference of, e.g., $+30^{\circ}$ leads to a shortening of the stance period of 0.0012s or 3.75%. The observed value is not in agreement with our prediction, which stated that the stance phase should be elongated on uphill walks (positive slopes). Obviously, there is no direct effect of gravity on stance period.

Walking uphill does not only require working against downward acceleration, the additionally produced force also needs to be transmitted to the ground, i.e. the animal needs to establish a certain grip between tarsi and substrate. In both cases, the production of maximum force and grip is a direct function of the number of legs that are at any time in contact with the ground. Would an ant by trying to avoid uncontrolled and possibly fatal translations adapt its walking behavior on an inclined surface by taking extra measures of security? Our fifth hypothesis thus claims that "*Ants adapt their gait coordination in order to produce more thrust and stronger surface attachment during uphill locomotion*". One way of keeping more feet on the ground is changing one's gait: Ants running on level ground employ a biphasic tripod gait. But insects can also use a metachron gait, which allows for more simultaneous foot-ground contacts and is therefore preferably used in climbing insects. The phase relationships of the legs were determined relative to the stepping cycle of the left middle leg (L2; phase=0: touch down of L2 initiating the stepping cycle analyzed, phase=1: subsequent toch down of L2 initiating the next stepping cycle). The mean phase shifts of the within-tripod legs are R1: 0.01±0.09 and 0.02±0.08 (*C. fortis* and *F. pratensis*, respectively); R3: 0.06±0.11 and 0.04±0.08. The legs of the alternate tripod show the following phase values: L1: 0.47±0.15 and

0.49±0.13; R2: 0.46±0.16 and 0.46±0.12; L3: 0.53±0.15; and 0.52±0.12. The phase of the front legs is positively influenced by speed (R1: α=3.78±0.66; L1: α=1.98±0.45 per m/s), i.e. with higher speeds the touch down of the front legs is delayed. The touch down of the ipsilateral hind leg (L3) however, happens earlier with higher speeds: α=–0.96±0.42. The same tendency, but with values of an order of magnitude lower, can be observed between species: The wood ants' front legs touch the ground later (α=0.39±0.10 and 0.18±0.07) while the ipsilateral hind leg (L3) touches the ground earlier (α=–0.22±0.06). There is no influence of individual body size or of slope on phase relationships. In conclusion, the ants remained faithful to their tripod gait throughout all steps analyzed but demonstrated an adaptation towards speed. There is no gait change at inclines of up to 60°.

Static stability can also be enhanced without gait transitions: The duty factor describes the fraction of the stance period in relation to the whole cycle period. The higher the (gait independent) duty factor, the longer a foot establishes ground contact. The steps analyzed reveal a duty factor of 0.52±0.08 for desert ants and 0.58±0.06 for wood ants. Speed is the major influence on duty factor (α≈–0.8 per m/s), i.e. at higher speeds the relative ground contact time diminishes. In wood ants, only the front leg shows a significantly higher duty factor than in desert ants (β=0.03±0.01). Individual body size and inclination have no influence on duty factor. In conclusion, safety parameters such as gait and duty factor are not influenced by slope. The locomotive system of both species of ants is very robust towards inclinations. The only parameter that varies greatly with inclination is speed of locomotion (Fig. 5.3). The fitted output parameters depend either on speed or on body size. Species effects occur mainly with respect to geometric parameters. Significant influences of slope occur mainly in temporal parameters but the effects are marginal.

Our results clearly show that the temporal parameters of ant locomotion are fairly robust against variations in slope. In addition, there is hardly any difference between the two species, and size effects are small. Hence, it is no surprise that the frequency of the whole stepping cycle behaves accordingly. In all three legs, stepping frequency correlates with speed (α≈56Hz per m/s), or in other words, speed is gained through a shortening of cycle period (α≈–0.22s per m/s). A significant species effect is found only in stepping period, but remains at a comparatively low value (β≈–0.004s in wood ants). Individual size influences the stepping frequency of the middle leg (δ=–1.21±0.65Hz per mm).

The influence of slope is significant on stepping period ($\delta \approx$–0.00006s per degree), and in the front leg it is significant on stepping frequency (δ=0.013$\pm$0.006Hz per degree). In both cases, the δ-values are close to zero and hence can be considered irrelevant. Even though stepping frequency and stepping period are reciprocals of each other, the goodness of fit-criteria, the log-likelihoods, differ considerably: The linear fits of the frequency and the stepping period produce log-likelihoods of $\approx$–2500, and $\approx$+2500, respectively. In a direct comparison with identical degrees of freedom (which is the case in our analysis), the higher value denotes a better fit. Hence, the stepping period allows for better linear fits than the stepping frequency. The slightly curve-like distribution of stepping frequency can also be seen in Figure 5.4.

5.3.2 Path integration on a slippery surface

As seen in the results of the kinematic study, ants do not adapt the kinematics of their walking behavior to the inclination of the surface on which they walk. However, as external forces act on the running ant, the production of force output has to vary, if a constant behavior is to be maintained on all inclinations. This seems to be the case according to the data presented above. The question remains, how path integration on inclines may be performed, assuming that idiothetic cues are responsible for inclinometry. If force production at a given speed is a function of inclination, force sensitive receptors could play an important role in path integration. Should one be able to break the relationship between force and step length experimentally, the ants should encounter difficulties in correctly estimating distances covered. Hence our sixth hypothesis will be the following: "*Desert ants running on a slippery surface will experience increased tarsal slipping and hence restricted force transmission to the ground. This effect eventually increased by external forces such as wind will cause the ants to misjudge their distances covered.*" As running on a slippery surface is a rather challenging task, for a man as well as the ant, we performed this behavioral study only on level ground. The foraging ants were either passively displaced backward by head wind or pushed forward by tail wind. At the same time the production of propulsive force was limited to the frictional properties of the substrate. And indeed, ants that experienced head wind during their outbound run produced a high number of short steps (see supplementary video, Suppl. 1). Although challenged by constant backward displacements the animals maintained

a highly coordinated gait pattern, which was qualitatively similar to locomotion on a rough substrate and resembled only temporarily the locomotion of water striders with front and hind legs resting and the middle legs "rowing" (e.g. Denny, 2004). When the ants were transferred to the test channel they still experienced the wind, but - now successfully clinging to the rough ground - did not encounter slipping during running. They expected the nest close to the position where the ants foraging under wind-free conditions searched for their nest (Fig. 5.7). The centers of the nest searches of the head wind ants (8.0±1.90m, n=18) did not differ from the centers of search of the wind free ants (7.23±1.22m, n=15) (p=0.39; Mann-Whitney U-test; Mann and Whitney, 1947). Compared to the zero control situation (outbound and inbound runs on rough ground, 9.1±1.4m), the wind free ants on slippery ground searched at an earlier stage of their inbound run (p=0.0005; U-test). On the other hand, if the ants experienced tail wind during their outbound runs, they were only required to perform very few steps which they also did in a highly coordinated manner (see supplementary video, Suppl. 2). Their estimation of the nest entrance was far shorter (4.15±2.46m, n=13) than that of the wind free control ants (p=0.0007; U-test). Under tail-wind conditions the ants underestimated the length of their outbound path. As each step on the micro-grit surface produced only a small and limited force, the number of steps required to reach the nest differed strongly with the experimental conditions. In consequence, the product of step and force is low in tail wind ants and high in head wind ants. This qualitative relationship is mirrored in the location of the centers of the search density distributions (Fig. 5.6)

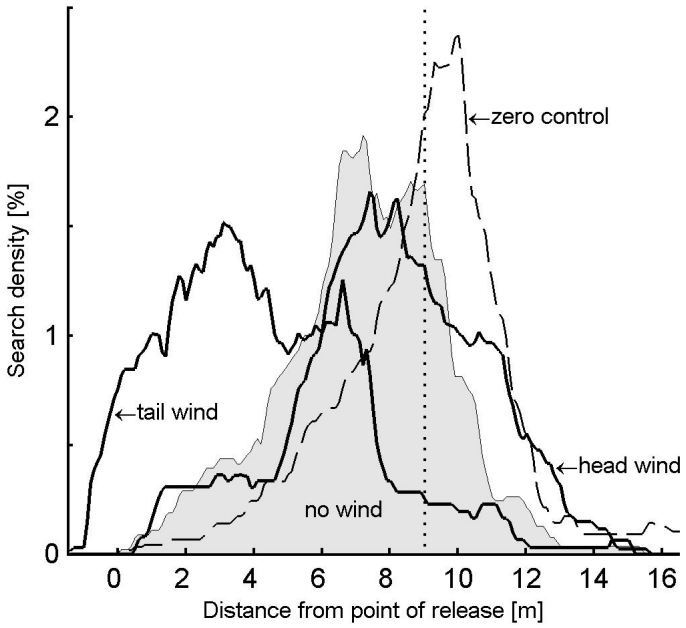

Figure 5.7: Density distributions of the ants' nest search behavior after the ants had performed outbound runs on slippery ground (except zero control ants) under different wind conditions (tail wind: n=13, no wind: n=15, head wind: n=18, zero control: n=20).

5.4 Discussion

The results from the kinematic analysis of ants running on inclines show that the ants employ a very robust locomotor behavior that is not influenced by the inclination of the substrate. The major influence slope has on kinematic parameters is speed of locomotion. Faster running ants make longer steps and do so at a higher frequency. This robust relationship, which had already been shown for *Cataglyphis* species walking on level ground (Zollikofer, 1988, 1994a, Wittlinger et al., 2007a), remains even if the ants are running on slopes.

5.4.1 Footfall geometry and step length

The footfall geometry of the tarsi, both in the anterior and posterior positions of the legs, differ mainly between the two species analyzed. This is a clear consequence of species-specific morphology. *Cataglyphis fortis* has comparatively longer legs than *Formica pratensis* has (Wehner and Sommer, in prep.). Hence *Cataglyphis* places its legs farther away from the body than *Formica* does. This difference in morphology allows the desert ant to make longer steps (see also: Zollikofer, 1994b). The values for step length on level ground obtained in the present study coincide well with the data presented by Zollikofer (1994a) for the same species. A similar effect can be found for individual body size as a covariant: larger animals make longer steps. The footfall geometry, however, is only partially affected by variations in body size. In conclusion, all geometric parameters are influenced by the morphology of the animals, and step length is additionally influenced by speed of locomotion. Contrary to the findings of Zollikofer (1994a), there is a slight influence of speed on some footfall parameters. This effect is mainly found in the middle legs: at higher speeds the middle tarsus is positioned closer to the body and hence can be placed forward at AEP (anterior extreme position) and prolonged at PEP (posterior extreme position). However, the effect is small and may have been below the accuracy of Zollikofer's experimental setup.

When walking on an inclined surface, the vertical projection of the animal's center of mass will shift considerably. Nevertheless an according shift of the footfall geometry, the support base of the stepping tripod, could not be observed. In conclusion, the ants either adjusted the body height in order do keep the vertical projection of the COM within the supporting base or they used their uphill legs to actively pull the body towards the surface. Force measurements on cockroaches running on inclined surfaces confirm that the uphill positioned legs generate negative force to keep the body close to the surface and hence to cling to the surface instead of "standing" safely (Goldmann et al., 2006). The apparent shift in weight distribution on the legs (while walking e.g. uphill, the hind legs will carry the majority of the body load) did not result in a leg-wise change in step length either in our study animals or in the cockroaches. The positioning of the legs including step length was not influenced by slope.

In the Introduction we assumed that the working area of the legs and as a consequence the footfall geometry and/or the resulting step length may be influenced by locomotion

on inclined surfaces. This proposed influence could be monitored by hair field sensors that occur at the leg joints. Although these joint-located hair sensors have been identified as graviceptive sensors in ants (Markl, 1962), our findings let us assume that measurement of the angular positions of the legs will not reveal gravimetric information. Similarly to the results of Wittlinger (2007a) these findings do not support the suggestions of Collett et al. (2006), who argued that the angular working area of the legs provides the animal with pedometer information.

5.4.2 Temporal coordination on inclines

The previously discussed geometric data obtained in our cinematographic studies on ant locomotion show that for monitoring the inclination of their walking floor ants do not exploit the static positions of their legs. However, the cyclic movements of their legs and the linear acceleration of the whole body are subject to external forces such as gravity and hence might be speeded up or slowed down during locomotion on inclined surfaces.

On level ground our results on temporal parameters such as swing and stance period, stepping frequency and phase relationships are - as the geometric parameters - in good accordance with previous kinematic studies on ant locomotion (Zollikofer, 1988). With higher speeds cycle time is reduced (or frequency is increased), mainly by a shortening of the stance phase. In other words, the duty factor, a measure of static stability, is reduced with speed. As ants run slower on inclined surfaces, especially on uphill paths, their duty factor is generally higher. We might speculate that if the ants choose to run slower on inclinations they do so for gaining higher stability than on level ground.

Leg coordination, i.e. the gait, remains constant on all inclines examined: The phase relationships of the legs clearly indicate that both species of ants employ an alternating tripod gait. The ants maintain their biphasic tripod gait, but show a small variability of leg coordination with speed. It is especially the front legs that touch down at a later moment within the cycle if the ants run faster.

The two species differ, but only to a small extent, in the temporal parameters of locomotion. In wood ants the cycle period is shorter. This is likely due to the shorter and hence lighter legs. However, this effect disappears when frequency instead of cycle

period is taken into account. These two fits are based on the very same data with one being the inverse of the other. But the model of cycle period has the by far higher log-likelihood-value and hence provides a better fit. From the data plotted in Figure 5.4 we conclude that frequency does not change linearly with speed, but cycle period does. Judging from the data plots the ants' maximum sustainable frequency lies in the range of about 25Hz for both species. The species-specific shortening of the stance period takes place at an even lower magnitude than the speed-specific reduction, and hence can be considered irrelevant. Bigger animals have a longer swing period. This may be a consequence of the higher angular momentum of a longer and therefore heavier leg. Again, this factor is an order of magnitude smaller than the speed-influences. Slope has a significant effect on cycle period and both stance and swing period, but again these values are small and therefore considered zero. The inclination of a substrate does not influence either the leg coordination or the different phases of the stepping cycle.

When running on an inclined surface, gravity has two effects on an animal's body: First, the body is pulled towards the surface on which the animal walks, but to a lower degree than if the animal were running on level ground; second, the animal's forward movement is either impaired (or supported) by the substrate-parallel fraction of the externally acting gravitational force. If the ant could not compensate for this external influence it would, on e.g. uphill runs, show a prolonged stance phase as active thrust is counteracted by downward acceleration or even a prolonged swing phase of the rather passively swinging legs. However, neither had been observed. The only effect that was observed lies in a variability of the relationship between step length and frequency in extreme situations of *Cataglyphis* locomotion: On steep uphill paths, when *Cataglyphis* is extremely slow, and on -30° downhill paths, when *Cataglyphis* runs rather fast, the step length at a given frequency do not coincide with the relationships of the other three conditions examined (Fig. 5.4a). This effect may be due to energetic conversion: While on the -30°-slope the conversion from potential energy to kinetic energy is supported and hence longer steps are possible, the production of sufficient potential energy during running on 60°-slopes impairs the ant from making long steps. However, this effect only occurs in *Cataglyphis* and disappears at the other slopes examined and might well be due to other effects, like e.g. individual body size. Hence it will not suffice for a reliable monitoring of inclination run. Joint position sensors, even when integrating over time, do not seem to qualify for inclinometric measurements employed by the ants in three-dimensional path integration.

5.4.3 Ants do not employ special kinematics

Previous studies on insect kinematics revealed two basic types of locomotion: a highly controlled feed-back type of slow locomotion and a fast feed-forward locomotion that relies rather on 'mechanical intelligence' than on sensory control (Full and Koditschek, 1999). The fast runners examined were mainly cockroaches (*Blaberus discoidalis*; e.g. Kozacik, 1981; Jindrich and Full, 2002), i.e. animals whose most elaborate navigational task is to escape bright light or approaching predators. On the other hand, the slow walking stick insects employ a highly elaborated control architecture that leads them through their extremely complex structured habitat (Cruse, 1976). Running on an inclination evoked an increase of duty factor in climbing locusts (Duch and Pflüger, 1995), and an elongation of the stance phase on uphill paths in cockroaches (stick insects: Cruse, 1976; cockroaches: Spirito and Mushrush, 1979; Delcomyn, 1985; Tang and Macmillian, 1986). In uphill running potato beetles *Leptinotarsa decemlineata* a statistically not significant decrease of stance period as well as an inclination-dependent variation of the footfall positions was observed (Pelletier and Caissie, 2001). While the the fast-running insects maintain constant locomotion patterns in virtually all environments coping with disturbances on a purely mechanic basis, the latter group employs highly variably kinematics in order to keep themselves on track.

Desert ants, however, have to meet contradictory demands: Their habitat provides shelters to heat and predators and hence, these animals have to employ both the high speeds of the cockroach (Jindrich and Full, 2002) as well as a high sensorial control over their movements as stick insects (Cruse, 1976), in order to get back to their safe nest in time. Now, which type of runner does the desert ants' locomotor system resemble? Or would we even see an intermediate type, allowing for high sensorial feedback at high speeds? Would probably the far-ranging desert ants in their open environment run differently than the landmark-following wood ants in their cluttered environment do (like in, e.g., arboreal and terrestrial lizards: Higham and Jayne, 2004)? Our results are clear: desert ants as well as wood ants employ the cockroach-type locomotion. Hence, this style of fast feed-forward locomotion still allows for sensorial monitoring of each single step, its corresponding length and the inclination of the substrate, although variations in the pattern of leg movements do not occur. The only influence the inclined surface has on the ant's walking parameters are the different speeds the animals choose to take.

The speeds recorded for *C. fortis* in the present account differ somewhat from the data obtained by Wohlgemuth et al. (2002) with ants foraging within a linear array of hills. In both studies *C. fortis* ants ran at about 0.2m/s (with maxima up to 0.4m/s) and reduced speed on uphill slopes, but to lower values in the latter study (0.05 vs. 0.1m/s at $\approx 60^{\circ}$). On downhill slopes, in either study the speed did not depend on the degree of the slope. In the present account the ants ran at 0.23m/s and hence twice as fast as the Wohlgemuth ants. The setup of Wohlgemuth et al. (2002) consisted of a series of hills which might have triggered the ants to run at lower speeds in order to save energy. The tendency to run slow on uphill slopes and to keep a fair speed of travel on downhill slopes is apparent in both studies. Energy recovery, i.e. the conversion of potential energy to kinetic energy, would allow the ants to save energy on downhill slopes. While potential energy recovery is well known in bigger animals (e.g. turkeys: Gabaldón et al., 2004), ants do not seem to use a substantial amount of the potential energy for propulsive means (Lipp et al., 2005).

5.4.4 Locomotion on slippery ground

The last hypothesis remaining for slope detection states that the ants measure forces that during walking act on their legs. Maintaining a constant movement pattern and hence compensating external influences indeed requires a variability in force production. When in our experiments the ants were running on a slippery surface this force production was limited and the step length was made variable by external (in our case wind-load) forces. The results of this pilot study show that ants, when running with strong head winds and hence producing many short steps integrate the very same nest-feeder distance as the wind free ants do. When foraging under strong tail-wind conditions, they will produce only very few but rather long steps and underestimate the nest-feeder distance considerably. Both head wind and wind free ants searched at an earlier stage than did the zero control ants. In contrast to the latter group the first two were confronted with different floor textures and colors on the channel ground during outbound and inbound situations. The experiments in chapter 3 revealed, among others, that inbound ants have an expectation of the visual and tactile ground properties and hence will have been influenced by the mismatch between a slippery white floor (outbound) and a rough light-brown floor (inbound) in the current study. Due to technical reasons it was not

possible to record an entire outbound run of ant ant in order to estimate the actually performed number of steps and the corresponding step lengths. However, the results at least qualitatively show that horizontally acting forces could be the desired correlate for step length, and that if the relationship between stepping force and step length is disturbed, the ants will miscalculate distances. As force sensors involved in graviception and kinesthetic orientation have been described in arthropod legs (insects: Horn, 1985; spiders: Seyfarth and Barth, 1972), we suggest that these should be the target of future experiments on the question on how navigating desert ants monitor step length and record surface inclinations.

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Supplementary material

Suppl. 1. *Cataglyphis fortis* foraging on micro-grit surface under head wind conditions. (Hardcopy: See attached CD; PDF: Double click icon to start movie in external player.)

Suppl. 2. *Cataglyphis fortis* foraging on micro-grit surface under tail wind conditions. (Hardcopy: See attached CD; PDF: Double click icon to start movie in external player.)

Appendix

Curriculum Vitae

Tobias Seidl

aus Deutschland

8. Oktober 1975 in Regensburg

Ausbildung

Mittelschule	Abitur, Ludwigsgymnasium Straubing, Deutschland (9/1986–6/1995) Leistungskurse: Biologie, Mathematik Schriftliches Nebenfach: Sozialkunde Mündliches Nebenfach: Deutsch
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Doktorat	bei Prof. Rüdiger Wehner, Zoologisches Institut, Universität Zürich (seit 3/2003)

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Project related publications

Peer-reviewed papers

2006 **Seidl T, Wehner R**. Visual and tactile learning of ground structures in desert ants. *Journal of Experimental Biology* 209:3336-3344.

Seidl T, Knaden M, Wehner R. Ant odometry: is active locomotion a prerequisite for path-integration? *Journal of Comparative Physiology A* **192**, 1125-1131.

Oral presentations

2006 **Seidl T, Weihmann T, Blickhan R, Wehner R**. Ants running on inclines: path integration and stability. (5th WCB-Congress (29.7.-4.8.2006) Munich, Germany) *Journal of Biomechanics* 39(Suppl. 1):S361.

Seidl T, Wittlinger M, Wehner R. Vertical object avoidance reaction in desert ants. (2005 Annual Main Meeting of the Society of Experimental Biology, Canterbury, UK) *Comparative Biochemistry and Physiology A* 143:S92.

Seidl T. Neuromechanik der dreidimensionalen Pfadintegration bei *Cataglyphis fortis*. PhD progress report. Project Meeting, Zurich University.

Seidl T. Short range navigation in desert ants. *CALZONE2*-Seminar, ETH-Zurich, Switzerland.

Seidl T. Neuromechanic mechanisms or short range navigation. *Oberseminar*, Dept. Neurobiology, Ulm University (invited talk).

2005 **Seidl T, Weihmann T, Blickhan R, Wehner R**. Kinematic analysis of ants running at different inclinations: walking stability and leg length. (Annual Meeting of the German Zoological Society, Bayreuth, Germany).

Seidl T. Neuromechanik der dreidimensionalen Pfadintegration bei *Cataglyphis fortis*. PhD-Progress report.

2004 **Seidl T**. Neuromechanik der 3D-Orientierung bei Ameisen. *Neurobiology Course*, Dept. Neurobiology, Inst. Zoology, Zurich University, Switzerland.

Seidl T. Estimating distance and inclination: a biomechanic approach. *Lunch seminar*, Dept. Neurobiology, Inst. Zoology, Zurich University, Switzerland.

2003 **Seidl T**. A functional analysis of some structures of the arthropod cuticle using atomic force microscopy. *Colloquium on neuro- and behavioral biology*, Dept. Neurobiology, Inst. Zoology, Zurich University, Switzerland.

Poster presentations

2007 **Seidl T, Wehner R**. Desert ants monitor substrate roughness while running. (Annual Meeting of the Society of Experimental Biology, Glasgow, UK) (accepted).

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